

## **Historic, Archive Document**

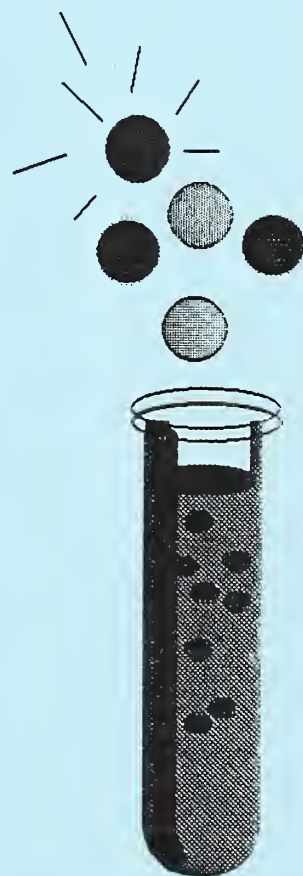
Do not assume content reflects current scientific knowledge, policies, or practices.



Reserve  
aQK710  
.2  
.P58  
1988

# PLANT STRESS LABORATORY REVIEW

1986 - 1987  
April 20, 1988



**United States  
Department of  
Agriculture**



**National Agricultural Library**

## TABLE OF CONTENTS

Agenda . . . . .	2
List of Invited Participants . . . . .	4
Current Mission Statement. . . . .	6
Major Resesarch Accomplishments. . . . .	7
List of Foreign Visitors . . . . .	11
Annual Operating Plan. . . . .	12
CRIS Allocation Schedule . . . . .	13
Position Staffing Chart. . . . .	15
High Priority Needs List . . . . .	16
Budget Increase Proposal . . . . .	18
Safety and Health Report . . . . .	19
Response to Recommendations from Previous Review . . . . .	20
Contributions from Scientists:	
Charles D. Foy. . . . .	22
Susan C. Miyasaka . . . . .	32
Alton L. Fleming. . . . .	34
Edward H. Lee . . . . .	38
Robert M. Zacharius . . . . .	50
Howard E. Heggstad . . . . .	54
Donald T. Krizek. . . . .	56
Robert K. Howell. . . . .	69
Charles R. Caldwell . . . . .	72
Merle M. Millard. . . . .	77
Eugene L. Vigil . . . . .	83
William P. Wergin . . . . .	92
Electron Microscopy Unit . . . . .	98

U.S.D.A., NAL  
 SEP 17 2002  
 Cataloging Prep



Agenda for Beltsville Area  
 Review of the Plant Stress Laboratory  
 Natural Resources Institute  
 April 20, 1988

8:00-8:10 a.m.	P. Kearney	Introduction
8:10-8:20 a.m.	W. P. Wergin	Laboratory overview
Mineral Nutrition CRIS NO. 1209-12000-001-OD		
8:20-8:40 a.m.	C. D. Foy	Soil-plant genotype interactions
8:40-8:50 a.m.	S. Miyasaka	Proposed program soil-plant interactions
8:50-9:10 a.m.	A. L. Fleming	Metabolic effects of environmental stress

Air Pollution  
 CRIS No. 1209-23000-010-OD

9:10-9:30 a.m.	E. H. Lee	Mode of action of pollutants
9:30-9:50 a.m.	R. Zacharius	Hypersensitive response
9:50-10:00 a.m.	H. Heggstad	Effects on growth and production
10:00-10:20 a.m.	Break	

Water Relations  
 CRIS No. 1209-23000-007-OD

10:20-10:40 a.m.	D. T. Krizek	Environmental physiology
------------------	--------------	--------------------------

Symbiotic Relationships  
 CRIS No. 1209-23000-009-OD

10:40-11:00 a.m.	R. K. Howell	Legume environment interactions
------------------	--------------	---------------------------------





## Biomembranes

CRIS No. 1209-23000-008-OD

CRIS No. 1209-23000-019-OD

11:00-11:20 a.m.	C. R. Caldwell	Regulation of membrane composition, structure and function
11:20-11:40 a.m.	M. M. Millard	Plant surface and membrane responses
11:40-12:00 p.m.	E. L. Vigil	Development and germination of seeds
12:00-12:20 p.m.	W. P. Wergin	Structure-function relationships in plant stress
12:20-12:30 p.m.	P. Kearney	Closing comments
12:30 p.m.	Executive Session (Closed)	



## List of Invited Participants

### External Reviewers

Dr. Bruno Quebedeaux  
Chairman, Department of Horticulture  
University of Maryland  
College Park, MD 20742

Dr. Charles Mulchi  
Department of Agronomy  
University of Maryland  
College Park, MD 20742

Dr. Robert P. Donaldson  
Biology Department  
George Washington University  
Washington, D.C. 20052

Dr. Kenneth Aycock  
Chairman, Department of Agronomy  
University of Maryland  
College Park, MD 20742

### National Program Staff

Dr. W. Doral Kemper  
Dr. Jerome P. Miksche  
Dr. A. Lawrence Christy  
Dr. Philip A. Miller  
Dr. Howard Waterworth

### ARS Scientists

Paul Terry, Plant Hormone Lab  
Chen Wang, Market Quality Research Lab  
Karl Norris, Instrumentation Research Lab  
Cathleen Somich, Pesticide Degradation Lab  
Dave Warthen, Insect Chemical Ecology Lab  
David Smith, Germplasm Services Lab



Lee Briggie, Germplasm Services Lab  
Tom Devine, Plant Molecular Lab  
Jack Murray, Germplasm Quality Enhancement Lab  
T. Austin Campbell, Germplasm Quality Enhancement Lab  
Robert Yaklich, Germplasm Quality Enhancement Lab  
Peter Semeniuk, Florist & Nursery Crops Lab  
J. R. Plimmer, Environmental Chemistry Lab  
A. Rango, Hydrology Lab  
S. L. Rawlins, Systems Research Lab  
G. F. Fries, Pesticide Degradation Lab  
G. Hart, Remote Sensing Research Lab  
D. D. Kaufman, Soil-Microbial Systems Lab  
W. VanDerWoude, Plant Photobiology Lab  
M. Faust, Fruit Lab  
D. L. Keister, Nitrogen Fixation & Soybean Genetics Lab  
Autor Mattoo, Plant Hormone Lab  
Lowell Owens, Tissue Culture Lab  
J. McBride, Information Staff  
S. Berberich, Public Affairs Specialist  
Staff and Affiliates of the Plant Stress Lab

#### Beltsville Area and Institute Personnel

Dr. W. Klassen, Area Director  
Dr. D. Murrell, Associate Area Director  
Dr. P. Kearney, Deputy Area Director, NRI  
Dr. J. Elgin, Deputy Area Director, PSI  
Dr. J. Menn, Associate Deputy Area Director, PSI  
Dr. D. Bills, Deputy Area Director, PQDI  
Dr. T. Sexton, Deputy Area Director, LPSI



Plant Stress Laboratory  
Natural Resources Institute

Beltsville Agricultural Research Center

PROBLEM:

Environmental extremes in soil, water and air decrease crop yields and limit production areas. As a result, the U. S. farmers experience increased costs and restricted productivity.

MISSION:

The mission of the Plant Stress Laboratory is to develop genetic and cultural methods and approaches that will broaden the adaptation of crop plants to extreme environmental conditions. Specific goals are: (1) to characterize - physically and chemically - crop plant responses to extremes of temperature, soil pH, minerals, water, air quality, light, and climatic interactions with biotic symbionts and antagonists; (2) to identify the existence of genetic variation in the response of crop plants and their wild relatives to stress; (3) to devise cultural methods of preventing or ameliorating the impact of stress on crop yield and quality; and (4) to isolate N<sub>2</sub> fixing combinations of legume-bacterial symbionts that are tolerant to environmental stresses.

<u>CRIS Nos.</u>	<u>Investigators</u>	<u>6 yr Plan</u>
1209-12000-001-OD	Foy, Fleming	1.2.02.1.A
1209-23000-007-OD	Krizek, Wergin	2.3.01.1.I
		2.3.01.1.H
1209-23000-008-OD	Caldwell, Wergin, Millard, Vigil	2.3.01.1.I
1209-23000-009-OD	Howell	2.3.01.1.D
		2.3.01.1.B
1209-23000-010-OD	Lee, Zacharius	2.3.01.1.I
1209-23000-019-OD	Millard	2.3.01.1.I





## MAJOR RESEARCH ACCOMPLISHMENTS

1209-12000-001-OD

C. D. Foy and A. L. Fleming

1. Found that Al stress decreased concentrations of citric, succinic, levulinic and total organic acids to a significantly greater degree in roots of Al-sensitive Kearney barley than in those of Al-tolerant Dayton. Similar HPLC evidence was obtained for Al-tolerant Dade and Al-sensitive Romano snapbeans. Evidence suggested that Al tolerance in these species may be associated with the complexation and detoxification of Al by naturally occurring or stress induced organic acids in plants. Evidence for this mechanism was less convincing for 5 wheat cultivars studied.
2. Found that Al toxicity and drought stress produced synergistic effects on the growth of Al-tolerant and sensitive cultivars of barley, sunflower and tomato grown in pots of acid, Al-toxic Tatum subsoil in a growth chamber. Such interactions must be considered in breeding plants for adaptation to acid soils.
3. Identified Al-tolerant strains of Old World bluestems (Genus Bothriochloa) and cultivars of oats. The bluestems show promise for use in reclaiming acid, marginal soils and producing forage at low cost. Al-tolerant oat cultivars may be used directly in acid soil rotations with potatoes in which soils must be maintained below pH 5.4 to control potato scab disease.

1209-23000-007-OD

D. T. Krizek and W. P. Wergin

1. Provided the first evidence for a synergistic effect of Al toxicity and water deficit on the growth of Al-tolerant and Al-sensitive cultivars of barley, sunflower, and tomato grown under controlled-environment conditions in an acid, Al-toxic Tatum subsoil. Such information is of vital importance in breeding and selecting cultivars that are adapted for growing on problem soils.
2. Established that, unlike water stress, root restriction had little or no effect on net assimilation rate in tomato. Although root restriction greatly reduced the total number and fresh and dry weight of mature fruits, plants grown in large-volume and small-volume containers had nearly the same percentage (41%) of total photosynthate in their reproductive portion after 12 weeks of treatment. These data suggest that for a given growing area, a culture system using small containers would be more efficient in producing fruit for a given size of plant than one using large containers. These findings have important implications for researchers and growers involved in growing plants in confined spaces.
3. Demonstrated the protective effect of low photosynthetic photon flux (PPF) pretreatment in ameliorating chilling injury. Plants exposed to 5°C for 48 to 72 h after pretreatment at  $8 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$  for 48 h showed less refreshed delayed light emission (RDLE) and chlorophyll fluorescence (FLU), contained more chlorophyll, and showed less chilling injury than those pretreated at  $320 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$  of PPF for 48 h.



4. In dose-response studies, found that uniconazole, was nearly 2-10 ten times more effective than paclobutrazol in providing protection against SO<sub>2</sub> injury in coleus and bean. Phytoprotection was obtained at lower uniconazole concentrations than those required to obtain maximum inhibition of stem elongation and leaf enlargement. These findings indicate that the phytoprotective effects of this triazole compound are not necessarily related to its growth-retarding properties as an anti-gibberellin.

1209-23000-008-OD      C. R. Caldwell, M. M. Millard, E. L. Vigil  
and W. P. Wergin

1. Utilized energy dispersive x-ray analysis, scanning and transmission electron microscopy to demonstrate that Fe<sup>+3</sup> reduction to Fe<sup>+2</sup> occurs at the outer surface of the plasma membrane of root hairs. This reduction is essential before iron can be taken up by root cells and transported within the plant.
2. Used cryostage on conventional scanning electron microscope. Showed that high vacuum evaporator can be used to shadow frozen tissue. Resolution obtainable, which is several-fold better than the conventional sputter coating technique that is commonly used, allows investigators to observe some macromolecular membrane structure on fresh, frozen, fractured biological tissue.
3. Examined Solanum nigrum isogenic biotypes that were resistant and susceptible to the herbicide atrazine. Study showed that chloroplasts from the resistant strain accumulated less starch than those from the susceptible (0.17 vs. 1.23 grains/section) and that grana lamellae in resistant chloroplasts were 20% longer than those from susceptible organelles. Study indicates that the single amino acid transversion in the psb-A gene which codes for the 32Kd protein also alters chloroplast structure-function relationships.
4. Applied new instrument, low voltage field emission scanning electron microscopy, to biological investigations. Showed that little or no coating allows resolution of biological structures that were not previously seen or described.
5. The data acquisition and analysis capability of the Dupont x-ray photoelectron spectrometer was updated by interfacing the data output from the Nicolet multichannel analyzer to a Compact 286 Desk pro mini computer. The system is also interfaced with an Epson FX286 printer and Hewlett Packard plotter. A data analysis software program was obtained from Dr. P. M. A. Sherwood, University of Kansas. This data analysis program allows a variety of operations to be performed on the data such as scale expansion, peak area measurement, least squares curve deconvolution and journal quality spectral plots.
6. Chemical and structural changes at the plant leaf surface interface region resulting from the action of SO<sub>2</sub> and ozone were detected using x-ray photoelectron spectroscopy and oxygen plasma etching. Damage processes such as surface oxidation, the accumulation of sulfate, potassium and calcium at the surface were identified.



7. Surface and structural chemical changes on tolerant and susceptible barley roots resulting from exposure to aluminum ion were detected and analyzed using x-ray photoelectron spectroscopy.
8. Impairment of protein body formation and loading due to environmental stress was determined for developing cotton seeds, using whole plant defoliation. Nutritional and drought stress has specific effect on population and content of protein bodies in the radicle cortex. This effect is proportional to duration of stress received. Study showed that protein body formation and maturation is critical to seed quality and germination.
9. Provided the first evidence for plant plasma membranes of the relationships between membrane lipid structure and protein conformation, and the function of membrane-bound enzymes.
10. Utilized a form of affinity chromatography not previously applied to membranes in the isolation of membrane proteins with differing affinities to potentially phytotoxic heavy metal cations.

1209-23000-009-OD

R. K. Howell

1. First to demonstrate that strains of Bradyrhizobium differ in their abilities to influence mineral content of peanut seeds and nodules.

1209-23000-010-OD

E. H. Lee and R. M. Zacharius

1. We have developed an in vitro system (e.g. cell and tissue culture) to study the basic mechanisms of ozone stress. We have examined free radical scavenger systems such as, ascorbic acid, alpha-tocopherol, superoxide dismutase, and other metabolites that may account for the variation in plant sensitivity responses to atmospheric pollutants, e.g., O<sub>3</sub> and SO<sub>2</sub>. Results have shown that the cellular protective system to ozone stress is linearly correlated to an increase of antioxidant compounds. These findings provide the backbone of future studies to explain the inherent differences of sensitivity to air pollutants. This should lead to a better understanding of defensive mechanisms associated with natural biochemical processes. The ultimate goal is to understand how gaseous pollutants function in order to minimize production losses and to maintain the integrity of both natural and agricultural plant communities.
2. We have shown that it is possible to increase the tolerance of plants to air pollution and temperature extremes by treatment with plant growth regulators. The data demonstrates that environmentally stress-induced injuries to crop plants may be reduced by the bioregulator; e.g., paclobutrazol and other antigibberellic acid compounds. The data also suggested that at least part of the protective mechanism with paclobutrazol treatment be attributed to its inhibition of gibberellic acid biosynthesis. We concluded that the gibberellic acids may play a role in determining plant sensitivity to SO<sub>2</sub>.





3. Experiments utilizing in vivo chlorophyll fluorescence measurements were conducted to monitor and screen  $O_3$  susceptibility. The experimental data suggest that fluorescence spectra provides a means of investigating pollution injury via photosystem I and II. The method appears to be suitable for the detection of gaseous pollutants.
4. Collaborative field experiments with the University of Maryland, Georgetown University, People's Republic of China, and USDA, on air pollution and insect interaction on vegetation were conducted. Results indicated that Mexican Bean Beetle (MBB) pupae weight increased when compared with the control, after feeding on  $SO_2$  and  $O_3$ -stressed foliage. Based on our experimental data and that of other workers, we have predicted that the crop damage from MBBs will escalate as air pollution increases in the future.
5. Developed a procedure for exposing discontinuous suspension cell cultures to air pollution stress or alternatively to free radical producing paraquat in media.





Foreign Scientists Who Have Worked in the Plant Stress Laboratory During  
the Past 2 Years

India:

Dr. S. N. K. Rao, FAO Fellow, Indian Institute of Horticultural Research, Bangalore, India. Visiting Scientist in lab for 2 months.

Prof. V. S. Rama Das, Head, Botany Department, and Dean, School of Biological and Earth Sciences, SRI Venkateswara University, India.

South Korea:

Dr. Ja Hyeong Ku, Korean Science Foundation fellow, Chungnam National University, Department of Horticulture, College of Agriculture, Daejeon, South Korea. Visiting scientist in lab for 1 year.

Japan:

Mr. Y. Kohno, Electric Power Industry (CRIEPI), Biology Dept., Abiko Research Lab., Japan. Visiting scientist in lab for 18 months.

China:

Wu, Yar, Jiangsu Academy of Agricultural Science, Institute of Plant Protection, Nanjing, PRC. Visiting scientist in lab for one year.

Brazil:

Mn. Nevio Nuernbert, Estado De Catarina, Secretaria Da Agricultura E Do Abastecimento, Empresa Catarinense De Pesquisa Agropecuaria, S.A. Estacao Experimental de Lages, Carta No 005/88, Lages, S.C. Brazil

Federal Republic of Germany:

Dr. Christa Lankes, Institut für Obstbau und Gemüsebau der Rheinischen, Friedrich-Wilhelms Universität, Auf dem Hügel 6 D-5300, Bonn 1, FRG, Visiting Scientist for 2 months.



OF: 02/19/88

## ANNUAL OPERATING PLAN

ANNUALIZATION LAPSE

801-1209-130

DOLLARS

FTE

12

NCY: 03

SCIENTIFIC

11,229

0.20

ID CODE: T5

MODE CODE

MANAGEMENT UNIT NAME

NON-SCIENTIFIC

5,489

0.20

03-10-12-1209-30-00-00-00

PLANT STRESS LABORATORY

TOTAL

16,718

0.40

THIS MU OCCUPIES  
ONLY FEDERAL SPACE

str. of Resources	POS CAT	OBJ CLS	Prior Year		Full Year		Difference		CRIS/IRC	
			Dollars	FTE	Dollars	FTE	Dollars	FTE	Distribution	
Personal Services									1209-12000-001-00	16.24%
Scientific Effort									1209-23000-007-00	9.29%
Research Scientist	1	1000	633,100	11.50	610,783	10.80	-22,317	-0.70	1209-23000-008-00	46.29%
Service Scientist	4	1000							1209-23000-009-00	9.14%
Support Effort									1209-23000-010-00	19.04%
Research Affiliate	2	6000								
Support Scientist	3	6000	178,600	6.00	186,025	6.00	7,425			
Technician/Aid/Asst	5	6000								
Specialist	6	6000								
Technician/Aid (Eng. & Sci.)	7	6000	138,950	6.30	159,422	7.00	21,372	0.70		
Trades & Crafts	8	6000	23,100	1.00			-23,100	-1.00		
Admin. Support	9	6000	69,000	3.00	43,227	2.00	-25,773	-1.00		
(Other)	0 & 6	6000								
vertime			5,000		5,000					
emium Pay										
omotion/Award/OSI/Merit Pay			13,490		8,000		-5,490			
PERSONAL - SUBTOTAL			1,060,340	27.80	1,012,457	25.80	-47,883	-2.00		
Level of Persons		2100	12,000		14,000		2,000			
Transportation		2200								
ent, Comm., Utilities		2300	12,000		12,000					
rinting & Reproduction		2400	4,000		8,000		4,000			
ther Services		2500	2,400		23,000		20,600			
Repair & Maintenance		2530			20,000		20,000			
Research Support Agreement		2554	30,000		15,000	0.20	-15,000	0.20		
Supplies and Materials		2600			190,063		190,063			
Contractual Services & Support		2000	35,000		40,000		5,000			
Equipment		3100			40,000		40,000			
Extramural		4000	42,680		15,000		-27,680			
ther										
ther										
LL OTHER - SUBTOTAL			138,080		377,063	0.20	238,983	0.20		
TOTAL			1,198,420	27.80	1,389,520	26.00	191,100	-1.80		
ASE FUNDS			1,198,420		1,349,520		151,100			
Management Criteria										
Percent in Salaries				88.48%		72.86%		-15.61%		
Support Years per SY				1.42		1.39		-0.03		
Bench Dollars per SY			104,210		128,655		24,449			
Discretionary Funds per SY			2,651		23,802		21,151			
Percent Discretionary				2.54%		17.98%		15.44%		
Percent Fixed Funds				97.46%		82.02%		-15.44%		

100.00

REMARKS: IRC = 264,493  
 ERS:ENCR=\$15,860;VAC=\$5,482;SWITCH=\$21,623;  
 RC=\$264,493;CHRGBKS=\$22,900;TEMP FUNDS FROM AD TO FBY \$40,000.

DATE

APPROVED SIGNATURE

W. Klassen

DATE

3-5-88

TELEPHONE NO.



## CRIS ALLOCATION SCHEDULE

CRIS No.: 1209-12000-001-OD - Differential plant tolerance to excess Al and Mn in acid soil and Fe deficiency in calcareous soils. .

LEAD SCIENTIST: Charles D. Foy

STRATEGIC PLAN APPROACH ELEMENT 1.2.02.1a  
2.3.01.1i

NET TO CRIS: \$225,614

SALARIES: \$206,544

% Salaries: 91%

CRIS NO.: 1209-23000-007-OD - Role of light, temperature and water stress on the chloroplast antioxidant system.

LEAD SCIENTIST: Donald T. Krizek

STRATEGIC PLAN APPROACH ELEMENT: 2.3.01.1i  
2.3.01.1h

NET TO CRIS: \$129,086

SALARIES: \$111,823

% Salaries 87%

CRIS NO.: 1209-23000-008-OD - Biophysical and biochemical properties of membranes in plant responses to environmental stress

LEAD SCIENTIST: Charles R. Caldwell

STRATEGIC PLAN APPROACH ELEMENT: 2.3.01.1i

NET TO CRIS: \$643,228

SALARIES: \$434,162

% Salaries: 68%

This CRIS has significant instrumentation costs associated with electron microscopy, x-ray photoelectron spectroscopy and the newly installed EPR.



CRIS NO.: 1209-23000-009-OD - Selection of stress resistant rhizobium and legume  
gene sources to increase N<sub>2</sub> fixation.

LEAD SCIENTIST: Robert K. Howell

STRATEGIC PLAN APPROACH ELEMENT: 2.3.01.1d  
2.3.01.1b

NET TO CRIS: \$127,028

SALARIES: \$92,821

% Salaries: 73%

CRIS NO.: 1209-23000-010-OD - Biochemical mechanisms of tolerance to air  
pollution and environmental stress in bean and  
tobacco plants.

LEAD SCIENTIST: Edward H. Lee

STRATEGIC PLAN APPROACH ELEMENT: 2.3.01.1i

NET TO CRIS: \$264,562

SALARIES: \$189,107

% Salaries: 71%





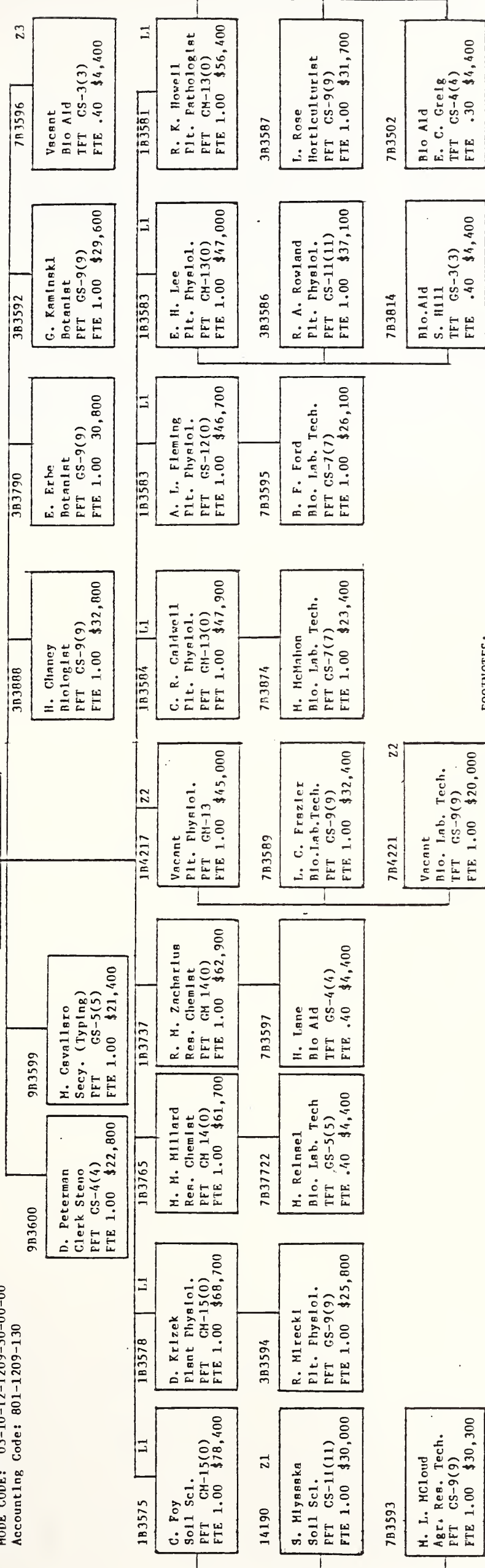
## 1.2

103577

## 1.2

W. P. Wergin  
Supv. Plt. Physiol.  
PFT GM-15(0)  
ETE 1.00 \$70.600

PLANT STRESS LABORATORY  
BELTSVILLE AREA  
MODE CODE: 03-10-12-1209-30-00-00  
Accounting Code: 801-1209-130



**FOOTNOTES:**

### 1.1.1 - Level 1 SV (i.e., Lead Scientist/Project Leader

1.2 - Level 2 SY (1.e., Research Leader)

Z1 - Salary Provided by Area Office

Z2 - Supported by New Funds (\$136,000), Accession

No. 0049423 In CRIS 1200-23000-009-00D

### 23 - Provide Technical Assistance for Collaborator



## HIGH PRIORITY NEEDS (Not priority listed)

1. LI-6200 LI-COR Portable Photosynthesis System Includes LI-6250 CO<sub>2</sub> Analyzer. System Console with 128K bytes memory, 9960-035 Sensor housing, two 60,000 TC spare leaf temperature thermocouples LI-190S-I Quantum Sensor, Battery Charger, & RS-232C output. This equipment is required for both lab and field studies on the effects of environmental stress on photosynthetic rate. There is no such equipment available in our Institute.

Approximate Cost - \$20,000

2. Micro-electrode equipment for determination of pH and electrical potentials at plant root-soil interfaces. Includes electrometer, microelectrode, micromanipulator and microscope. Equipment will permit elucidation of root-soil interface reactions in regulating differential Al and Mn tolerance in plants.

Approximate Cost - \$15,000

3. Support for a cooperative project between the Univ. of MD for air pollution field research. Air pollution field research requires a critical mass of scientific expertise and labor, as well as solid funding to be successful. We have good facilities at Beltsville but no supporting funds to operate.

Approximate Cost - \$50,000/year

4. Transparent rigid molded plastic isolators to investigate plant responses in germfree environments. This requested unit will enable us to culture cells of intact plants under axenic environments so that we can determine the specific effect of one stress factor on the host.

Approximate Cost - \$20,000

5. Furnishing and remodeling of Bldg. 050, Room 13C. Divide this room into two laboratories. The remodeling of this room is essential for the investigation of the physiological and biochemical nature of differential tolerance of selected genotypes to environmental stress.

Approximate Cost - \$150,000

6. Environmental control chambers and tissue culture chambers. Replacement or upgrade of three walk-in growth chambers and one tissue culture incubator in Bldg. 050 to provide precise control of light, temperature, and relative humidity.

Approximate Cost - \$140,000



BUDGET INCREASE PROPOSAL

Additional funding is particularly needed in 1209-12000-001-0D and 1209-23000-007-0D. The current permanent funding levels of these two units are not sufficient to support ongoing research. Net to CRIS should be increased by about \$30,000 and \$20,000, respectively, to bring these CRIS units to a salary/all other ratio of about 80/20.

In addition, funding for service contracts and support personnel in the Electron Microscope Unit would greatly alleviate the constraints in 1209-23000-008. Although this unit serves many laboratories at BARC, it is not always feasible nor practical to recover funds for service and/or cooperative research efforts.



SAFETY AND HEALTH

The Safety and Health Inspection of the Plant Stress Laboratory was conducted as required by ARS Directive 230-1, Occupational Safety and Health Program - Policy Authority, on April 6, 1988, by Mr. John Taylor, NRI. The Area Safety Officer is not participating in inspections. Inspection form ARS-404 is on file and indicates that there were no serious safety or health hazards resulting from laboratory operations.





## Response to Recommendations From Previous Review:

## 1. Recommendation:

The major problem for the laboratory is funding. There is no apparent source for fund increases this year, but the lab must be made solvent. The Area will work with the Institute and National Program Staff to develop options.

## Response:

Funding has improved during the past two years as a result of transfers, retirements and \$136,000 new funds. PSL has lost 3 SYs and 2 technicians; however, we have only replaced 1 SY in order to improve our salary/all other ratio which is 73/17 in our 1989 ARMPs.

## 2. Recommendation:

The lab should look carefully at its space. If space is not being used effectively (for example where unreliable or unused growth chambers are located) then that space should be released for use by others. This will reduce overhead next year.

## Response:

Greenhouse, storage and laboratory space which no longer served the needs of PSL was surplus in FY 1987. However, a recent SY hire, 2 new post-docs and a visiting scientist anticipated in FY 89 have increased our needs for both office and quality laboratory space.

## 3. Recommendation:

Regrettably, the lack of funding has resulted in the inability to hire full-time support for four scientists. When scientists do not have technical help, productivity declines. The Research Leader should use his best management judgment to assign technical help where it is most likely to result in the greatest increase in final output, i.e., publications and new discoveries.

## Response:

Reassignments within the laboratory have partially solved this problem. However, two scientists remain without the services of full-time technicians. 1040 appointments are being used to support these programs until further improvements in productivity and finances justify additional support.



#### 4. Recommendation:

In some instances, investigators may have to narrow the scope of their research objectives. This can be advantageous in that it will allow the investigator to focus on one or more closely related areas and allow a deeper understanding of the process or processes under study.

#### Response:

This matter has been discussed with the scientists who are encouraged to cooperate with other SYs at BARC whenever a broad scope is necessary to achieve their research objectives. As a result, several scientists in PSL have achieved international recognition in their fields and multiple authorship papers in PSL are the general rule.

#### 5. Recommendation:

Cooperation with industry is encouraged. Scientists in the laboratory are encouraged to increase their interaction with industry. Participants from industry are encouraged to attend the lab review.

#### Response:

Cooperation with industry remains our most elusive goal. Individuals from other user groups such as EPA, NASA, Corps of Engineers, NIH, NCI, DOE, NAS, Smithsonian Institute, U.S. Army Institute of Dental Research, Johns Hopkins, etc. do attend our workshops and seminars and seek expertise from our scientists. Two instrumentation manufacturers (from England and Japan) are very much interested in the Electron Microscope Facility and have provided on consignment at no charge expensive instruments in order that their potential to biological research could be assessed. However, the laboratory does not currently have any formal research and development agreements or patents with industry.

#### 6. Recommendation:

Publications are a final product of ARS research and are one of the measures used to evaluate the productivity of scientists. Scientists should discipline themselves to get results written up for publication. Even if they are without technical help, it is essential that after an experiment is completed, the scientist stop, write up the results, and reassess the situation before designing the next experiment.

#### Response:

This recommendation has been addressed to the SYs and is evaluated biannually through the individual performance standards. Present standards require a minimum of one senior authored or two co-authored manuscripts/yr in order to attain a "fully successful" rating.



- I. Name : Charles D. Foy  
 Title: Research Soil Scientist  
 Grade: GM-15
- II. CRIS Project No.: 1209-12000-001-OD  
 Title: The Physiology of Differential Plant Tolerances to Aluminum and Manganese Toxicities in Acid Soils and to Iron Deficiency in Calcareous Soils.
- III. Current Research Objectives and Progress (1986-87) and Plans (1988)
- A. Overall Objective: The long range objective is to exploit plant genetic potentials in solving soil fertility problems that are not economically correctable with current technology (tailor the plant to fit problem soils). The major emphasis is on the identification of physiological and biochemical "ear marks" of genetically controlled mineral stress tolerance that may be useful in selecting or breeding superior plants for better adaptation to problem soils. The major mineral stresses emphasized are Al and Mn toxicities in acid soils and Fe unavailability in calcareous soils.
- B. Objective: Identify and quantify naturally occurring (or stress induced) ligands (organic acids, amino acids, proteins, etc.) that may complex and hence, detoxify excess Al or Mn within the root zone or within the plant (wheat, barley, cotton, snapbean, soybean, sunflower, oats, Old World bluestems).
- Progress: Aluminum stress decreased concentrations of citric, succinic, levulinic and total organic acids to a significantly greater degree in roots of Al-sensitive Kearney barley than in the those of Al-tolerant Dayton. HPLC evidence also indicated that Al treatment reduced concentrations of organic acids, particularly citric and malic, to a greater degree in Al-sensitive Romano snapbean than in Al-tolerant Dade. This evidence suggested that Al tolerance in barley and snapbean may be associated with the complexation and detoxification of Al by naturally occurring or stress induced organic acids in plants. Differential Al tolerances among five wheat cultivars were not closely associated with differences in concentrations of total organic acids in plant shoots; however, under Al stress, the Al-tolerant Atlas 66 cultivar showed a 32% increase in citric acid concentrations in shoots while the Al-sensitive Scout was unaffected in this regard. Hence, it appears that concentrations of organic acids (inherent or Al-induced) are less indicative of Al tolerance in these cultivars of wheat than in those of barley and snapbean studied earlier. Preliminary electrophoresis indicated that Al stress decreased protein (mol. wt. 40 k d) production to a greater degree in Al-sensitive Kearney barley than in Al-tolerant Dayton. Naturally occurring or stress-induced proteins may complex and detoxify Al in tolerant plants.





Plans: Refine electrophoresis approach to determine the identities and concentrations of naturally occurring or stress-induced (by Al or Mn) ligands associated with stress tolerance in plant cultivars. Newly acquired ion exchange chromatography equipment will be used for greater precision in detecting organic acids than would be possible by conventional HPLC.

- C. Objective: Characterize the physiology of Al x drought x plant cultivar interactions in snapbean, barley, sunflower and sorghum (may include studies of enzymes and of hormones such as IAA, cytokinins and abscisic acid).

Progress: Aluminum toxicity and drought stress produced synergistic effects on the growth of Al-tolerant and sensitive cultivars of barley, sunflower and tomato grown in pots of acid, Al-toxic Tatum subsoil in a growth chamber. Such interactions must be considered in breeding plants for adaptation to acid soils.

Plans: Continue studies to elucidate the physiology of Al x drought x plant genotype interactions. Collaborative efforts are planned to demonstrate the role of Al tolerance in drought avoidance under field conditions.

- D. Objective: Determine the possible roles of Al x P, Al x Ca and Al x Si interactions in differential plant tolerances to Al.

Progress: Collaborative NMR studies showed that Al stress decreased concentrations of glucose 6 phosphate, inorganic P (cytoplasm and vacuole) and 3 species of ATP in live root tips of Al-sensitive Scout wheat to a greater extent than in those of Al-tolerant Atlas 66. This was the first "in vivo" characterization of Al toxicity in terms of Al x P interactions. Such information may be helpful in understanding the biochemistry of Al tolerance and in breeding Al-tolerant plants for adaptation to acid soils. Susceptibility to hypocotyl necrosis in snapbean and blossom end rot in tomato (both Ca deficiency disorders) coincided with greater tolerance to acid, Al-toxic Tatum subsoil.

Plans: Two NMR units at Beltsville (one new unit, plus one updated) will be used to determine the location, solubility and chemical form of P in live tissues of Al-tolerant and sensitive cultivars of several species. In related studies, the micro-imaging unit on the new NMR instrument and EM techniques will be used to characterize structural abnormalities induced by Al in tolerant vs sensitive cultivars.





- E. Objective: Characterize differential Mn tolerances (in pairs of tolerant and sensitive cultivars of cotton, soybean and wheat) in terms of Mn uptake, distribution and chemical forms (oxidation states) occurring in plants.

Progress: Evidence to date indicates that Mn tolerance in cultivars of wheat, soybean and cotton is associated with higher internal Mn tolerance rather than reduced Mn uptake. Differential Mn tolerances in two cotton genotypes, discovered earlier in high Mn acid soil, were confirmed in nutrient solutions containing excess Mn. Superior Mn tolerance in the C-310-73-307 genotype (compared to Sgl-70-517) was associated with a consistently higher Fe/Mn ratio in leaves.

Plans: Use newly acquired EPR instrument to characterize Mn tolerance in terms of the oxidation state of this element in tolerant and sensitive plant cultivars containing comparable concentrations of Mn. Possible Mn detoxifying roles of Fe and Si will also be examined.

- F. Objective: Characterize Mn tolerances in terms of stomatal activity and leaf temperature changes in soybean.

Progress: Mn toxicity in soybean was characterized by reduced stomatal conductivity and increased leaf temperature. This effect was significantly greater in Mn-sensitive Forrest than in Mn-tolerant Lee. Superior Mn tolerance in Lee was associated with higher internal tolerance to Mn and not with reduced Mn uptake. Mn sensitivity in Forrest was not associated with low Ca or Fe concentrations in leaves. Drought stress in soybean produced greater increases in leaf temperature than did Mn toxicity, but the effects were similar in the two cultivars. These findings may have application in geo-prospecting or crop assessment by remote sensing.

- G. Objective: Investigate soil-root interface reactions in relation to Al and Mn tolerance and to Fe efficiency.

Progress: Aluminum tolerance in wheat cultivars has been associated with pH increases in soil and nutrient solutions, higher nitrate reductase activity, higher  $\text{NO}_3^-$  uptake and lower  $\text{NH}_4^+$  uptake.

Plans: Use electron emission spectroscopy to determine distribution of Mn, Al and other ions on root surfaces. Use microelectrodes to determine pH changes in root mucigel layers. Use EM, NMR and X-ray microprobe to characterize stress tolerant and sensitive plants with respect to structure and function of root plasma membranes, the development of root hairs and the production of mucilage.



- H. Objective: Collaborate with plant breeders in the identification, breeding and physiological characterization of superior plant genotypes for use on problem soils.

Progress: Species and strains of Old World bluestems (Genus *Bothriochloa*) differed significantly in tolerance to excess Al in acid Tatum soil and in nutrient solutions. Tolerant strains show promise for use in reclaiming acid, marginal soils and in producing forage at low cost. They may also be useful as breeding materials in designing plants to fit specific soils. Oat cultivars from regional nurseries differed by 2 to 3 fold in tolerance to acid, Al-toxic Tatum soil. Tolerant cultivars may be useful in reducing damage by Al toxicity in acid soil rotations such as oats and potatoes in soils which must be kept below pH 5.4 for control of potato scab disease. Eleven Polish oat cultivars differed in tolerance to excess Al in nutrient solutions and in acid soils. Superior Al-tolerance was associated with significantly higher N concentrations in grain. Results suggested that selection for acid soil tolerance may increase N fertilizer efficiency in oats. Seven weed species differed significantly in tolerances to Al-toxicity, Mn toxicity and salinity. Results may be useful in weed control or in the use of weed species for reclamation of acid, saline or alkaline soils. H. N. Lafever (Wooster, Ohio), an early collaborator who released 'Titan' wheat which has moderate Al tolerance, has now released 'Cardinal' wheat which is more tolerant to Al than Titan and also out-yields standard cultivars on non-acid 'good' soils in Ohio. Like Titan, Cardinal can be used in an acid soil rotation with potatoes. A modified mass selection approach produced a significant increase in Al tolerance within the R123 strain of *Amaranthus cruentus*. Results suggested Al tolerances in this strain may be directly related to some desirable agronomic properties.

Aluminum tolerant BH 1146 and Al-sensitive Sonora 63 wheat cultivars could be separated on acid, Al-toxic Tatum subsoil under the following conditions: pH (1:1 soil-water) less than 5.2; KCl extractable Al levels greater than 2 c moles kg<sup>-1</sup> and Al saturations greater than 20% of CEC. Hence, any soil test used to predict Al-toxicity in acid soils must take into account the Al tolerances of the plant cultivars involved.

Plans: Collaborate with breeders in search of germplasm having superior tolerance to Al and Mn toxicities. Species involved include sorghum, soybeans, wheat and alfalfa.



#### IV. Cooperators:

- Dr. Edward Lee, Mr. A. L. Fleming, Dr. D. T. Krizek, Dr. Charles Caldwell, Dr. Merle Millard, Dr. William Wergin, Dr. Susan Miyasaka and Miss Carol Coradetti, Plant Stress Laboratory, ARS, Beltsville, MD.
- Dr. James Duke, Narcotics Laboratory, ARS, Beltsville, MD.
- Mr. Jack Murray and Dr. Austin Campbell, Germplasm Quality Enhancement Laboratory, Beltsville, MD,
- Dr. Tom Devine, Plant Molecular Laboratory, Beltsville, MD.
- Dr. D. H. Smith and Dr. L. W. Briggie, Germplasm Services Laboratory, Beltsville, MD.
- Dr. David Warthen (NMR). Insect Chemical Ecology Laboratory, Beltsville, MD.
- Dr. Cathleen Somich (NMR), Pesticide Degradation Laboratory, Beltsville, MD.
- Dr. R. R. Duncan (sorghum breeder) and Dr. Lee Ramseur (Plant Physiologist), Univ. of Ga., Expt. Sta., Griffin, GA.
- Dr. W. A. Berg and Dr. C. L. Dewald, USDA-ARS, Woodward, Oklahoma.
- Dr. Nevio Nuernberg, Lages, S.C., Brazil.
- Dr. J. J. Bilski, Agr. & Techn. Univ., Soil Sci. Dept. 10-2-27, OLSZTYN-KORTONO, Pl. 40, Poland.
- Dr. Andrzej Aniol, PL-ARS-118 (Fg-Po-369) "Biochemical and Genetical Aspects of Aluminum tolerance in Cereals". Plant Breeding and Acclimatization Institute, Radzikow, Poland, 3 yr. project approved 8/31/87.
- Dr. V. C. Baligar, USDA, ARS, Beckley, WV.
- Dr. Thomas Carter (soybean breeder), NC State Univ., Raleigh, NC.

#### V. Curriculum Vitae:

##### A. Educational History:

- 1949 - B.S. Univ. of TN, Knoxville; Major - Agriculture (Agronomy)
- 1953 - M.S. Purdue Univ., West Lafayette, IN: Major - Soil Science.
- 1955 - Ph.D. Purdue Univ., Major - Soil Fertility, minors in Plant Physiology and Chemistry.



B. Post Graduate Employment History:

1955-57 - Asst. Prof. Agronomy (Research), Purdue Univ., W. Lafayette, IN.  
 1957-61 - Soil Scientist, GS-11 to GS-12, USDA, ARS, SWC, Univ. of Arkansas, Fayetteville, Arkansas.  
 1961-72 - Soil Scientist, GS-12 to GS-14, U.S. Soils Lab., USDA, ARS, Beltsville, MD.  
 1972-present - Soil Scientist, GS-14 to GS-15, Plant Stress Laboratory, Beltsville, MD.

C. Society Offices Held: None

Membership in Professional Societies:

American Society of Agronomy  
 Crop Science Society of America  
 Soil Science Society of America

D. Editorial Board Appointments: None

E. Advisory and Consultant Appointments:

1. Advisory:

Graduate advisor for R. Suresh, Geology Dept., Univ. of Md., M.S. 1987.  
 Graduate Advisor for Carol Coradetti, Agronomy Dept., Univ. of Md., current M.S. candidate.  
 Co-investigator on PL-480 project, PL-ARS-118 (FG-P0-369) "Biochemical and Genetical Aspects of Aluminum Tolerance in Cereals", Plant Breeding and Acclimatization Institute, Radzikow, Poland. Principal investigator - Dr. Andrzej Aniol.  
 Advisor to Dr. J. J. Bilski, Agr. & Techn. Univ., Soil sci. Dept. 10-2-27, OLSZTYNI-Kortono, PL 40, Poland (acid soil tolerances of plants).  
 Advisor to Dr. Susan Miyasaka, post doctoral visitor (Al x plant genotype interactions at soil-root interfaces).  
 Cooperative research with several plant breeders and physiologists in developing and characterizing Al and Mn tolerant and Fe efficient plants (see list of cooperators).

2. General Consulting Activities:

Regular consultant to the U.S. Army Corps of Engineers in the revegetation of hostile sites (dredged material, road cuts, etc.).

3. ARS and BARC Activities - None since 1986.







F. Awards:

- 1987 - Elected Fellow in Soil Sci. Soc. Amer.  
 1987 - Received Merit Pay plus a performance cash award for an outstanding research rating of 5.0.

G. Other Significant Scientific Impact:

- Member, Organizing and Editorial Committee for X Intl. Plant Nutr. Colloq., August, 1986, Rockville, MD.  
 Invitational participant in Intl. Symp. on "Acidification in Tropical Countries", Caracas. Venezuela (1986) (Expenses paid).  
 Keynote speaker on "Al and Mn Phytotoxicity" for Symp. "Plant-Soil Interactions at Low pH", Grand Prairie, Alberta, Canada (1987) Expenses paid.  
 Accepted invitation to make presentation and write chapter on "Manganese Toxicity" for Intl. Symp., Waite Institute, Glen Osmond, Australia (1988). Expenses paid.  
 Accepted invitation to speak on "Al Toxicity" at Auckland, New Zealand, in connection with Australian Mn conference in 1988. Expenses paid.

VI. PublicationsA. Peer Reviewed Publications (1985-88)

1. Taylor, G. J. and C. D. Foy. 1985. Mechanisms of aluminum tolerance in wheat (Triticum aestivum L.). I. Differential pH in the rhizosphere of winter wheat cultivars. Amer. J. Bot. 72:695-701.
2. Taylor, G. J. and C. D. Foy. 1985. Mechanisms of aluminum tolerance in wheat (Triticum aestivum, L.). II. Differential pH in the rhizospheres of spring wheat cultivars. Amer. J. Bot. 72:702-706.
3. Taylor, G. J. and C. D. Foy. 1985. Mechanisms of aluminum tolerance in wheat (Triticum aestivum, L.). III. Long term pH changes in the rhizospheres of winter wheat cultivars differing in tolerance to aluminum. J. Plant Nutr. 8:613-628.
4. Taylor, G. J. and C. D. Foy. 1985. Mechanisms of aluminum tolerance in wheat (Triticum aestivum, L.). IV. The role of ammonium and nitrate nutrition. Can. J. Bot. 63:2181-2186.
5. Taylor, G. J. and C. D. Foy. 1985. Differential uptake and toxicity of ionic and chelated copper in Triticum aestivum L. Can. J. Bot. 63:1271-1275.



6. Taylor, G. J. and C. D. Foy. 1985. Effects of aluminum on the growth and element composition of 20 winter cultivars of Triticum aestivum L. (wheat) grown in nutrient culture. J. Plant Nutr. 8:811-824.
7. Foy, C. D., W. A. Berg and C. L. DeWald. 1987. Tolerances of old World bluestems to an acid soil high in aluminum. Plant and Soil 99:39-46.
8. Lee, E. H. and C. D. Foy. 1986. Aluminum tolerances of two snapbean cultivars related to organic content evaluated by high performance liquid chromatography. J. Plant Nutr. 9:1481-1498.
9. Foy, C. D. 1987. Acid soil tolerances of two wheat cultivars related to soil pH, KCl - extractable aluminum and degree of aluminum saturation. J. Plant Nutr. 10:609-623.
10. Bilski, J. J. and C. D. Foy. 1987. Differential tolerances of oat cultivars to aluminum in nutrient solutions and in acid soils of Poland. J. Plant Nutr. 10:129-141.
11. Campbell, T. A. and C. D. Foy. 1987. Selection of grain Amaranthus species for tolerance to excess aluminum in an acid soil. J. Plant Nutr. 10:249-260.
12. Suresh, R., C. D. Foy and J. R. Weidner. 1987. Effects of excess soil manganese on stomatal function in two soybean cultivars. J. Plant Nutr. 10:749-760.
13. Baligar, V. C., J. R. Wright, T. B. Kinraide, C. D. Foy and J. H. Elgin, Jr. 1987. Aluminum effects on growth and mineral uptake and deficiency ratios in red clover cultivars. Agron. J. 79:1038-1044.
14. Foy, C. D., E. H. Lee and S. B. Wilding. 1987. Differential aluminum tolerances of two barley cultivars related to organic acids in their roots. J. Plant Nutr. 10:1089-1101.
15. Foy, C. D., D. H. Smith, Jr. and L. W. Briggles. 1987. Tolerances of oat cultivars to an acid soil high in exchangeable aluminum. J. Plant Nutr. 10:1163-1174.
16. Bilski, J. J. and C. D. Foy. 1988. Differential tolerances of weed species to aluminum, manganese and salinity. J. Plant Nutr. 11:93-105
17. Krizek, D. T. and C. D. Foy. 1988. Role of water stress in differential aluminum tolerance of two barley cultivars grown in an acid soil. J. Plant Nutr. 11. (Accepted 1/20/88.)



18. Krizek, D. T. and C. D. Foy. 1988. Mineral element concentration of two barley cultivars in relation to water deficit and aluminum toxicity. *J. Plant Nutr.* 11: (Accepted 1/20/88.)
19. Krizek, D. T., C. D. Foy, and W. P. Wergin. 1988. Role of water stress in differential aluminum tolerance of six sunflower cultivars grown in acid soil. *J. Plant Nutr.* 11: (Accepted 1/20/88.)
20. Krizek, D. T. and C. D. Foy. 1988. Mineral element concentration of six sunflower cultivars in relation to water deficit and aluminum toxicity. *J. Plant Nutr.* 11: (Accepted 1/20/88.)
21. Foy, C. D. 1988. Plant adaptation to acid, aluminum toxic soils. Invitational speech and chapter for Symp. "Plant Soil Interactions at Low pH," Grande Prairie, Alberta, Canada, July 20-24, 1987. *Comm. Soil Sci. Plant Anal.* 19:7-12.
22. Campbell, T. A., J. H. Elgin, Jr., C. D. Foy and J. E. McMurtrey, III. 1988. Selecting alfalfa for aluminum tolerance. *Can. J. Plant Sci.* (Accepted).
23. Suresh, R., C. D. Foy, J. R. Weidner, C. S. Schnetzler and M. Schwaller. 1988. Effect of mineral stress on the thermal infrared emission of soybeans: A greenhouse experiment: Possible utility in remote sensing. *Intl. J. Remote Sensing.* (Submitted.)
24. Suresh, R., C. D. Foy, and J. R. Weidner. 1988. Effects of water stress (drought) and manganese stress (toxicity) on two cultivars of soybeans: Possible applications in remote sensing. In review for *J. Plant Nutr.*
25. Baligar, V. C., R. J. Wright and C. D. Foy. 1988. Differential responses of forage legumes to aluminum. *J. Plant Nutr.* (In review).

#### B. Book Chapters

1. Kamprath, E. J. and C. D. Foy. 1985. Lime fertilizer - plant interactions in acid soils. "Fertilizer Technology and Use", Third Edition, Soil Sci. Soc. Amer., Madison, WI.
2. Foy, C. D., B. J. Scott and J. A. Fisher. 1988. Invitational speech and chapter for Intl. Symp. "Manganese in Soils and Plants," Waite Agr. Res. Inst., Adelaide, South Australia, August 22-26, 1988. To be published in *Proc. by Martinus Nijhoff.* (Submitted).



C. Other Publications and Abstracts:

1. Taylor, G. J. and C. D. Foy. 1985. A possible role of nitrogen nutrition and rhizosphere pH in the aluminum tolerance of cultivars of Triticum aestivum, L. In Lekkas T. D. (ed.) Intl. Conf. on "Heavy Metals in the Environment", Athen, Greece. Conf. Proc. Vol. 1, pp. 319-321.
2. Taylor, G. J. and C. D. Foy. 1985. The role of chelation in metal tolerance and toxicity; differential uptake and toxicity of ionic and chelated copper in Triticum aestivum L. In Lekkas, T. D. (ed.), Intl. Conf. on "Heavy Metals in the Environment", Athen, Greece, Conf. Proc., Vol. 1, pp. 316-319.
3. Murray, J. J. and C. D. Foy. 1985. Developing aluminum tolerant tall fescue strains for acid soils. pp. 843-844, Proc. 5th Intl. Turfgrass Research Conf., Avignon, France (Abstract).
4. Foy, C. D., M. Farina and A. J. Oakes. 1985. Iron-manganese interactions in strains of Nile grass (Acroceras macrum, Stapf.), Agron. Abstracts, p. 171, Amer. Soc. Agron., Madison, WI.
5. Nuernberg, N. J., C. A. Bissani, C. D. Foy, M. Dall'Agnol and T. A. Campbell. 1988. Screening pasture plants for aluminum tolerance. Abstract for Third Intl. Symp. on "Genetic Aspects of Plant Mineral Nutrition" to be held in the Institute of Crop Science and Plant Breeding, Federal Research Centre of Agriculture (FAL), Braunschweig, Federal Republic of Germany, June 19-23, 1988.
6. Millard, M. M. and C. D. Foy. 1988. Investigation of aluminum ion stress in barley roots by X-ray photoelectron spectroscopy. Abstract for annual meeting of Amer. Soc. Plant Physiol., Reno, Nevada, July 10-14, 1988.
7. Krizek, D. T., C. D. Foy, N. K. Strinvosa Rao, C. Coradetti and R. Mirecki. 1988. The role of water stress in differential aluminum tolerance of two tomato cultivars differing in drought tolerance. Abstract for annual meeting of the Amer. Soc. Plant Physiol., Reno, Nevada, July 10-14, 1988.





I. Name: Susan C. Miyasaka  
Title: Soil Scientist, Post-doctoral  
Grade: GS-11

II. CRIS Project No.: 1209-12000-001

III. Projected Research Objectives (1988):

- A. Objective: To determine whether the control of rhizosphere pH is a mechanism for the avoidance of Al toxicity by plants.

Plans: A cooperative research project is planned with Dr. Leon Kochian of the U.S. Plant, Soil, & Nutrition Lab. at Cornell University, to measure rhizosphere pH, membrane potentials, and  $K^+$  fluxes in cultivars of wheat and barley that differ in Al tolerance.

- B. Objective: To determine whether the release of root exudates is another mechanism for the avoidance of Al toxicity by plants.

Plans: Cultivars of snapbean and barley that differ in Al tolerance will be grown aseptically, and root exudates will be analyzed for organic acids, using the Dionex ion exclusion chromatograph.

- C. Objective: To study the effects of Al toxicity on root hair growth and development in cultivars that differ in Al tolerance.

Plans: A misting chamber, or glass-sided soil boxes will be used to produce copious root hair growth. Snapbean and barley cultivars that differ in Al tolerance will be grown, and root hair growth will be compared in the presence or absence of aluminum.

IV. Curriculum Vitae:

A. Educational History:

1988 - Ph.D. Agronomy; Cornell University, Ithaca, NY.  
(Minors in Plant Physiology and Agricultural Engineering)  
1979 - M.Sc. Agronomy and Soil Science; University of Hawaii,  
Honolulu, HI.  
1976 - B.Sc. Entomology; University of California - Berkeley,  
Berkeley, CA.  
1971 to 1973 - Attended Kalamazoo College, Kalamazoo, MI.



B. Post-graduate Employment History:

- 1988 to present - Post-doctoral Soil Scientist, GS-11, at USDA - ARS Plant Stress Lab., Beltsville, MD.
- 1985 to 1987 - Graduate Research Assistant, at Cornell University, Ithaca, NY.
- 1983 to 1985 - Graduate Fellow, at Cornell University, Ithaca, NY.
- 1980 to 1983 - Agronomist/Soil Scientist, at BioEnergy Development Corp., Hilo, HI.
- 1977 to 1979 - Student Lab. Technician, at the Soil and Plant Tissue Testing Lab., University of Hawaii, Honolulu, HI.

C. Honors and Awards:

- Gamma Sigma Delta, The Honor Society of Agriculture, 1987.
- Bradfield Award, Cornell University, Dept. of Agronomy, 1986.
- Phi Kappa Phi, Honor Society, 1985.
- Sigma Xi, The Scientific Research Society, 1985.
- Cornell Graduate Fellowship for Continuing Students, 1984-85.
- Sage Graduate Fellowship, Cornell University, 1983-84.

V. Publications:

A. Peer Reviewed Publications:

1. Miyasaka, S.C., R.T. Checkai, D.L. Grunes and W.A. Norvell. 1988. Methods for controlling pH in hydroponic culture of winter wheat. Agron. J. (in press).
2. Yost, R.S., D.S. DeBell, C.D. Whitesell and S.C. Miyasaka. 1988. Early growth and nutrient status of Eucalyptus saligna as affected by nitrogen and phosphorus fertilization. Aust. For. Res. (in press).

B. Other Publications and Abstracts:

1. Miyasaka, S.C. 1988. Factors influencing calcium accumulation by winter wheat forage (Triticum aestivum L.): pH buffering, temperature, and nitrate levels. Ph.D. thesis, Cornell University. 226 pp.
2. Miyasaka, S.C. and D.L. Grunes. 1987. Root temperature effects on root growth and K, Ca and Mg in wheat forage. Agron. Abstr. American Society of Agronomy, Madison, WI. pp. 210.
3. Miyasaka, S.C., R.T. Checkai, D.L. Grunes and W.A. Norvell. 1986. Methods for controlling pH in hydroponic culture of winter wheat. Agron. Abstr. American Society of Agronomy, Madison, WI. pp. 208.



- I. Name : Alton L. Fleming  
Title: Plant Physiologist  
Grade: GS-12
- II. CRIS Project No.: 1209-12000-001-OD  
Title: The Physiology of Differential Plant Tolerances to Aluminum and Manganese toxicities in Acid Soils and to Iron Deficiency in Calcareous Soils.
- III. Current Research Objectives and Progress (1986-87) and Plans (1988):
- A. Objective: To determine the physiological basis of the regulation of Fe-stress response in legumes. Specifically, 1) to evaluate the extent to which the accumulation of Mn is regulated by the intensity of Fe deficiency and 2) to define the biochemical components of the system, responsible for the enhanced uptake of Fe, Mn and Zn.
- Progress: Mn-sensitive bean cultivars, which showed enhanced accumulation of Mn under Fe stress, were more responsive to Fe deficiency than Mn-tolerant cultivars, as indicated by increases in the reductive capacity of the root, and H-ion efflux and a higher enhancement index (EI) for Mn.
- Plans: To develop techniques for the isolation and measurement of components of the plant system responsible for the enhanced uptake of Fe, Mn and Zn.
- B. Objective: To determine the range of response among bean cultivars to nutrient and environmental conditions which can result in Fe deficiency. These considerations include assessment of the impact of N source, P and bicarbonate on the uptake and distribution of Fe, Mn, Zn.
- Progress: Sensitivity to Mn was characterized by enhanced Mn accumulation in the leaves, increased total Mn and toxicity symptoms on lower leaves when the plants were grown under Fe stress. Mn tolerant cultivars accumulate proportionately greater amounts of Mn in the roots. These cultivars may also possess a greater ability to resist high concentrations of Mn in the leaves. Changes in the distribution of Mn and Zn in the plant tissues reflected the cultivar response to low available Fe in the nutrient solution.
- Plans: To determine whether the distribution patterns of Mn and Zn in Fe-deficient snapbeans can be altered by changes in the concentration of micro-nutrients or in the intensity of Fe deficiency.
- C. Objective: Evaluate interaction of N metabolism and spectral quality on the development of chlorosis in chrysanthemum and snapbean cultivars.



Progress: Application of a fertilizer containing reduced N (urea and  $\text{NH}_4$ ), induced chlorosis of the upper leaves of soil-grown snapbean plants grown under low pressure sodium lamps (LPS) in growth chambers. Similar injury occurred when plants were grown in nutrient solutions, containing less than 10%  $\text{NH}_4\text{-N}$ . The chlorosis was evident on leaves which expanded after the LPS exposure and was characterized by severe bleaching of the leaf without obvious necrosis.

Plans: To quantify the influences of the form of available N to the plant and Fe metabolism on the chlorosis induced by exposure to LPS radiation. Leaf symptoms induced with LPS and Fe deficiency will be compared using chemical and EM microanalytical techniques.

#### IV. Cooperators:

Dr. Donald T. Krizek, Plant Stress Laboratory, ARS, Beltsville, MD.  
 Dr. Charles D. Foy, Plant Stress Laboratory, ARS, Beltsville, MD.  
 Dr. William P. Wergin, Plant Stress Laboratory, ARS, Beltsville, MD.

#### IV. Curriculum Vitae:

##### A. Education History:

1952-57 Howard University; major, chemistry; minor, zoology; B.S. 1957.  
 1968-74 University of Maryland; major, Agronomy; minor, Plant Physiology, M.S. 1984.  
 1958-59 Howard University; 8 semester hours Advanced Inorganic and Analytical Chemistry; 4 semester hours Physical Chemistry.  
 1960-61 National Bureau Standards Graduate School; 6 semester hours of mathematics.  
 1964 N.I.H. Graduate school; 3 semester hours of mathematics.  
 1965-66 USDA Graduate School; 6 semester hours of biochemistry.  
 1974 USDA Graduate School; Reading Improvement course (18 hours).  
 1977 MIT, Cambridge, Mass.; summer session short course (40 hours), Ultrasound, Laser, Ultraviolet and Microwaves.

##### Military Education:

1958 U.S. Armor School; Armor Officer's Basic Course (Certificate).  
 1968-73 U.S. Armor School; Officer's Advanced Course (Certificate).  
 1973-74 Command General Staff college, Phases I, II, and III. Present Rank: Major (USAR-Retired).







B. Post-Graduate Employment History:

1960 GS-4, Physical Science Aid, USDA, Beltsville, MD.  
 1960-64 GS-5, Physical Science Technician, USDA, ARS,  
 Beltsville, MD.  
 1964-66 GS-7, Physical Science Technician (Chemistry), USDA,  
 ARS, Beltsville, MD.  
 1967-69 GS-9, Chemist (Research Assistant) USDA, ARS,  
 Beltsville, MD.  
 1969-75 GS-11, Chemist, USDA, ARS, Beltsville, MD.  
 1975-present GS-12, Plant Physiologist, USDA, ARS,  
 Beltsville, MD.

C. Society Officers Held:

None

D. Editorial Board Appointments:

None

E. Advisory and Consultant Appointments:

1. Advisory:

Collaboration with other members of the Plant  
 Stress Laboratory on projects of mutual interest.

2. General Consulting Activities:

Conducted seminars for science classes at local secondary  
 schools, 1985-1988.

Participated as a guest lecturer for Natural Resources  
 course at Delaware State College, 1987.

3. ARS and BARC Activities:

Member of organizing and editorial committee for the Tenth  
 International Plant Colloquium, 1986, Beltsville, MD.

F. Awards:

Elected to membership in Phi Sigma Society, 1974.

G. Other Significant Scientific Impact:

Invited seminars: None 1985-88.

Invited Symposiums: None 1985-88.



Publications:

A. Peer Reviewed Publications:

Fleming, A. L., Krizek, D. T., and Mirecki, R. M. Influence of  $\text{NH}_4$ -nutrition on the growth and mineral composition of two chrysanthemum cultivars differing in drought tolerance. J. Plant Nutr. 10(9-16):1869-1881.

Krizek, D. T., Mirecki, R. M., Fleming, A. L., and Dubik, S. P. LPS-induced chlorosis in chrysanthemum as influenced by genotype and ammonium/nitrate ratio. J. Plant Nutr. 10(9-16):1059-1069.

B. Book Chapters:

None

C. Other Publications and Abstracts:

Fleming, A. L., Krizek, D. T. and Mirecki, R. M. Influences of  $\text{NH}_4$  nutrition on the growth and mineral composition of two chrysanthemum cultivars differing in drought tolerance. Abstracts of the Tenth International Plant Nutr. colloq. (page 24), Beltsville, MD., Aug. 4-9, 1986. (Abstract).

Krizek, D. T., Mirecki, R. M., Fleming A. L. and Dubik, S. P. Differential  $\text{NH}_4$  toxicity in chrysanthemum as influenced by spectral quality,  $\text{NO}_3$  and  $\text{NH}_4$  nutrition. Abstracts of the Tenth International Plant Nutr. Colloq. (page 18), Beltsville, MD, Aug. 4-9, 1986 (Abstract).

Fleming, A. L. Micronutrient accumulation and distribution in Fe deficient snapbean plants. Agronomy Abstracts (page 199) 1986 Annual Meeting of the American Society of Agronomy, New Orleans, LA. 1986.

Krizek, D. T., Fleming, A. L., Mirecki, R. M. and Dubik, S. P. Growth and development, chlorophyll content and mineral composition of tomato as influenced by  $\text{NH}_4$  and  $\text{NO}_3$  nutrition and exposure to water stress. Abstracts of the Tenth International Plant Nutr. Colloq. (page 24), Beltsville, MD, Aug. 4-9, 1986. (Abstract).



I. Name: Edward H. Lee

Title: Plant Physiologist

Grade: GM-13

Technical Assistance: Randy Rowland (Support Scientist--Plant Physiologist).

II. CRIS No. : 1209-2300-010

Title : **Biochemical Mechanisms of Tolerance to Air Pollution and Environmental Stress in Bean and Tobacco Plants**

III. Current Research Objectives and Progress (1986-87) and Plans (1988).

- A. Objective 1: Determining the biochemical mechanisms associate with air pollutants and assessing the nature of its susceptibility.

Progress: 1. Relationships between foliar ozone ( $O_3$ ) tolerance and leaf ascorbic acid (AA) concentrations in  $O_3$ -susceptible ( $O_3$ -S) 'Hark' and  $O_3$ -resistant ( $O_3$ -R) 'Hood' soybean cultivars were examined by high-performance liquid chromatography (HPLC). Results indicated that superior  $O_3$  tolerance in the 'Hood' cultivar (compared with 'Hark') was associated with increased endogenous levels of AA which may scavenge free radicals and thereby protect cells from injury by  $O_3$  or other oxyradical products.

2. Experiments were conducted to examine the effects of an experimental chemical, [paclobutrazol (PBZ)] on sensitive crop cultivars, and its relation to plant resistance under  $SO_2$ , chilling, and heat stress. After a brief PBZ treatment (0.2 mg/15-cm pot), two environmentally sensitive cultivars of snapbeans were afforded significant protection against visible injury when exposed to high concentrations of  $SO_2$  (1.5 ppm for 3h) or temperature stress (4 °C/24 h or 40 °C/72 h) in laboratory studies. The  $SO_2$  tolerance induced by PBZ was reversed by subsequent application of  $GA_3$ . The data showed that the susceptibility of sensitive plants to  $SO_2$  stress can be manipulated by treatment with this growth regulator and plant hormone,  $GA_3$ . These findings may be of interest to the plant growth regulator industry in the development of programs and practices for adverse environmental stress situations.

3. An in vitro system was utilized to study the mechanisms of ozone tolerance and phytotoxicity in plant tissue culture. Nicotiana tabacum L. cultures, one sensitive (cv. 'Bel W-3') and one tolerant (cv. 'Bel B') to  $O_3$ , significantly decreased fresh and dry weights of both clones; however, this decrease was greater in cell line 'Bel W-3' than in 'Bel B'. Suspension cell cultures of S. tuberosum cv. 'Kennebec' and Glycine max cv. 'Mandarin' in aqueous media required higher  $O_3$  levels to elicit responses. Peroxidase levels increased in both species at 40 ppm. At 10 and 40 ppm  $O_3$ , the superoxide dismutase levels of the potato cells were sharply lowered but





to a much lesser degree than the soybean culture. The stress metabolite, glyceollin, was induced in soybean cultures with 40 ppm  $O_3$ , but was not observed at 10 ppm. In addition, our suspension culture experiments failed to support the hypothesis that phytotoxicity of de novo formed stress metabolites, such as phytoalexin compounds, were responsible for damage of tobacco tissue induced by  $O_3$  stress.

**Plan 1:** Variation in plant sensitivity to air pollutants may result from several factors including stomatal physiology, photosynthesis inhibition, free radical scavenging systems, photosynthate allocation, or cellular response mechanisms. At present, the mechanism(s) responsible for differential plant sensitivity and its relationship to plant growth and productivity are not well understood. The mechanism of antioxidant-induced resistance against air pollutants remains unclear. The members of NE-121, the regional Hatch project entitled "Reducing the Influence of Air Pollution on Plant Productivity to the Northeast" met at the University of Massachusetts in January of 1988. The outcome of the gathering was the formation of a new research working group entitled "Ethylene Diurea (EDU) Air Pollution Working Group". As members of the EDU Working Group we are interested in evaluating systemic fungicides, antioxidants, and growth regulators by two-fold: one, to evaluate their ability to protect plants against photochemical oxidants, and secondly, to explore the mechanisms of successful antiozonants. An antioxidant compound, (EDU) will be used as a research tool to elucidate the chemical protection mechanisms. Our specific research interests are to explore the nature of ozone toxicity, as well as, the mechanism(s) of EDU-induced resistance to ozone. Experiments will be conducted to analyze the effects of ozone and EDU-induced changes in the cellular membrane phospholipid components, and sterol contents of bean (Phaseolus vulgaris cv. 'Bush Blue Lake 290' (BBL- 290) first trifoliate leaves. Plants will be exposed to selected  $O_3$  or  $SO_2$  concentrations which produce visible damages ranging from slight to severe. The structural component of cell membrane phospholipids will be analyzed by gas chromatography or HPLC. Experiments will be conducted to determine if the levels of individual sterol and membrane phospholipids in bean leaves can be correlated with air pollution resistance.

**Plan 2 :** Previous studies have shown that plants vary in their susceptibility and resistance to air pollutant stresses. Polyamine may play a role in the protection against or detoxification of free radicals in plants. Therefore, elevation of endogenous polyamine levels in response to  $O_3$  and  $SO_2$  stress will be examined. Although polyamines have been modeled for various plant processes, there is little evidence for their involvement in the free radical scavenging process. Therefore, the regulatory role of polyamines in response to  $O_3$  or  $SO_2$  stresses in plant tissues should be examined. Endogenous levels of the polyamine putrescine, spermidine, and spermine in the first trifoliate leaves of two cultivars of





bush bean 'BBL-290' and 'Astro' which differ in their response to  $O_3$  stress will be quantified with HPLC. Relative amounts or changes in polyamine content will be correlated with  $O_3$  tolerance.

- B. Objective 2: Identifying and screening crop plants tolerant to  $O_3$  and  $SO_2$ .

Progress: The utilization of chlorophyll fluorescence (Chl-F) yield as a screening tool for assessing  $O_3$  tolerance in cultivars of snapbean (*P. vulgaris* L.) was investigated. Ozone-stressed leaves showed significantly higher constant yield ( $F$ ) but greatly reduced variable fluorescence ( $f_v$ ) and maximum  $F_v/F_{max}$  ratios. In the  $O_3$ -S cultivar a strong inhibition of the fast and slow fluorescence induction transients, altered the form of the kinetic curves of Chl-F in leaves. In particular, the fluorescence quenching rate and  $F_v/F_{max}$  ratios were markedly decreased in  $O_3$ -stressed leaves. In contrast, leaves of the  $O_3$ -R 'Astro' showed only minor changes in Chl-F. The values of the ratio  $F_v/F_{max}$  decreased in the  $O_3$ -S cultivar more drastically than in the  $O_3$ -R cultivar.

The significant finding of the Chl-F measurement technique was that it can be used as a diagnostic tool for detecting the differential tolerance of snapbean to  $O_3$  stress. With the potential to be further developed for testing plant resistance to other environmental stress factors.

- C. Objective 3: To determine mechanisms of tolerance of plant cultivars to air pollutant interactions with biotic and abiotic factors.

Progress 1. In cooperative field studies between the Plant Stress Laboratory, USDA-ARS, and the Agronomy Department at the University of Maryland conducted in 1984 and 1985 were completed during 1987. Four cultivars of wheat were exposed from one week preanthesis until maturity (June) to six air pollution treatments. Vegetative samples were collected weekly from ten days post anthesis until grain maturity. Changes in grain loading rates and chemical contents were examined in an effort to explain observed changes in yields, seed weights, and harvest index caused by  $O_3$  stress. Seed growth and grain fill rates were retarded by the highest  $O_3$  stress treatments. Seed carbohydrate composition, expressed as mg/g was largely unchanged; however, sucrose, glucose, fructose, and starch contents, expressed as mg/seed, were significantly reduced by the Non-filtered air + 40 and 80 nLL  $O_3$  treatment.

Progress 2: Two field investigations were conducted in 1987 which involved the effects of  $O_3$  stress and the elevation of  $CO_2$  concentrations on soybean. Both studies were part of a cooperative agreement between the Plant Stress Laboratory, USDA-ARS, and the Agronomy Department at the University of Maryland.



Yield results from both studies are being analyzed and chemical analysis of samples has been initiated. In general, the Washington DC area was subjected to drought during the months of July and August when samples were being collected. Although the plots were irrigated frequently, there was evidence that drought stress may have affected the results. Also, severe heat may have affected the  $C^{13}$  uptake studies. Both studies are scheduled to be repeated during the 1988 growing season.

**Progress 3:** Collaborative field and laboratory experiments with the University of Maryland, Georgetown University, the People's Republic of China, and Plant Stress Laboratory, USDA/ARS, were conducted in 1986 and 1987, to examine the interaction of air pollution, plants, and insects. The objectives of the experiments were: 1) to determine if growth of Mexican Bean Beetles (MBBs) was affected by feeding on intact plants of snapbean exposed to  $SO_2$  or  $O_3$ . 2) to determine if direct exposure of the insects to the pollutants alters their growth and development. 3) to explore the possible cause(s) of MBB feeding preferences on soybeans. Results for **Objective 1:** both sexes grew significantly larger on  $O_3$ -treated plants compared to controls (males, a mean of 18% greater than controls, females, 16%). **Objective 2:** males grew significantly larger (15%) on  $SO_2$ -treated foliage compared to controls. Females feeding on  $SO_2$ -treated foliage and both sexes feeding on  $O_3$ -treated foliage did not grow significantly larger than controls. Comparison of both experiments suggests that direct  $O_3$  fumigation of MBB larvae decreases their pupal weights. **Objective 3:** suggests that MBBs consumed  $O_3$ -stressed leaves in preference to NF or CF treated leaves. The  $O_3$ -stressed leaves had a significantly higher concentration of non-structural carbohydrates than non-stressed leaves.

These findings support previous results obtained by other workers, and further suggest that crop damage from MBBs will increase as air pollution increases in the future.

**Plan:** Assessment of the interaction between  $O_3$  and  $CO_2$  on soybean will be conducted in the summer of 1988 and 1989. The project with the University of Maryland will be continued and it will be closely linked with our air pollution research. The project will study the effects of  $O_3$  stress on photosynthate partitioning in plants, physiological mechanisms associated with cultivar sensitivity to  $O_3$  stress, and interaction of low-level  $O_3$  stress with  $CO_2$  on water relations in plants during mild moisture stress. This study will be conducted in cooperation with a professor and graduate students from the University of Maryland. The experiments will be performed on the South Farm, USDA/ARS, BARC-West, where similar studies using open-top chambers, have been conducted in previous years.



IV. Cooperators:

- a. Plant Stress Laboratory, USDA/ARS: Drs. M. M. Millard, C. D. Foy, R. Zacharius, D. T. Krizek.
- b. Plant Hormone Laboratory: Dr. R. Saftner
- c. Plant Photobiology Laboratory : Dr. Steven Britz
- d. University of Maryland-- Dr. C. Mulchi, field study on effects of  $O_3$  on soybean.
- e. Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, People's Republic of China-- Mr. Yar Wu, on Effects of air pollution on the growth of Mexican Bean Beetles.
- f. Georgetown University--Dr. E. M. Barrows, on air pollution, plant, and insect interactions.
- g. Department of Horticulture, Chungnam National University, Daejeon, South Korea-- Dr. Ja Hyeong Ku, On efficiency of XE-1019 as a phytoprotectant against  $SO_2$  injury in snapbean.
- h. Florist and Nursery Crop Laboratory, USDA/ARS,-- John W. Neal Jr.

V. Curriculum Vitae:A. Educational History:

1959 - B.S. National Taiwan University. (Botany). 1966 - M.A. University of Kansas, Lawrence, Ks. (Plant Physiology).

1969 -Ph.D. University of Oklahoma, Norman, Ok. (Plant Physiology - Biochemistry).

Post Doctoral: 1970 Illinois Institute of Technology (Biochem.)

1973 summer months, Univ. of Saskatchewan--(Tissue culture).

Training Courses:

1971 Argonne National Laboratory (Radioisotopes Workshop).

1973 Summer, Univ. of Alabama, (Molecular Biology).

1980 HPLC Short course--Water Associates.

1983 80 hrs Plant Molecular Biology, USDA/Graduate School.

1985 40 hrs. USDA Supervisory Skills and Management training.

1986 40 hrs. SAS Course, Complex Statistical Procedures, USDA.





## B. Professional Positions:

1961-64. Laboratory Instructor, taught Plant Physiology, General Botany & Systematic Botany, at the Dept. of Botany, National Taiwan University.

1964-66. NIH Research Assistant, Univ. of Kansas, Lawrence, Ks.

1966-69. Teaching Assistant, University of Oklahoma, Norman, Ok.

1969-78. Associate Professor of Biology, taught Comparative Cellular Biology & Biochemistry, Microbiology, Genetics and Plant Physiology courses at Central Methodist College, Fayette, Missouri.

1974-76. (Summer months): Visiting Research Professor, Department of Biological Science, Univ. of Missouri Columbia, Missouri. (Research on electron transport systems & photosynthesis).

February 1978-June 1982. GS-11, Plant Physiologist, USDA/ARS, Plant Stress Laboratory, PPHI., Beltsville, Maryland.

July 1982-August 85. GS-12/13, Plant Physiologist, Plant Stress Laboratory, USDA/ARS, Beltsville, Maryland.

June 1985-present. Adjunct Associate Professor, Dept. of Agronomy, Univ. of Maryland.-College Park, Maryland.

September 1985-present. GM-13/14 Plant Physiologist, Plant Stress Laboratory, USDA/ARS, Beltsville, MD.

## C. Membership in Professional Societies:

American Society of Plant Physiologists

Air Pollution Working Group

Tissue Culture Association

North America Phytochemistry Society.

Plant Growth Regulator Society.

Sigma Xi

North America Taiwanese American Professor Association

## D. Offices and Committee Assignments held in professional and honorary Societies:

President, North America Taiwanese American Professor Association Washington/DC & Baltimore Chapter.

Chairman, Taiwanese American Conference/ East Coast, 1988.

Chairman, NAPA Constitution Revision Committee, 1987.





Member, Greenhouse Committee, BARC, ARS. 1986-87.

**E. Advisory and Consultant Appointments:**

Graduate Advisor for Leon Slaughter, Wayne Spencer, and Roman Pausch, Ph.D. candidates, Agronomy Department, Univ. of Maryland.

To serve as a doctoral thesis outside viewer and examiner for Ms. Catherine Whitende, Zoology Department, Howard University.

To serve as science advisor for high school students; Sherita Hill, L. Linsey, K. Holland, and Gloria Kim. 1985-87.

To serve on Local Arrangement Committee, North American Phytochemical Society's Annual Meeting, 1986.

Advised visiting scientists, educators, and industrial representatives, for contacts within ARS, seeking information and recommendations concerning air pollution effects on vegetation and cleansing of the atmosphere.

Served to review manuscripts for the Journals of Plant Physiology, Environmental & Experimental Botany, Environmental Quality, etc.

Served as a reviewer to evaluate a research proposal for a USDA/Competitive Research Grants.

**F. Honors and Awards:**

- |         |  |
|---------|--|
| 1964-66 | NIH Research Fellowship awarded by the University of Kansas.   |
| 1966-69 | Research Assistantship awarded by the University of Oklahoma.  |
| 1971    | Recipient of NSF College Faculty Research Grant Award.   |
| 1971    | Recipient of Outstanding Educator of America award given by National Educator of American Organization (Nominated by the President of the Central Methodist College, Fayette, Missouri). |
| 1974-76 | Recipient, NSF visiting professor Research Grant, University of Missouri-Columbia, Mo.   |
| 1975    | Recipient of Outstanding Educator of America (Nominated by the Dean of College, CMC)   |



- 1976            Awarded as a tenure professor by the president of the college at Fayette, Missouri.
- 1970-78        Appointed by the president of the college as Chairman of Pre-med Advisory Board, CMC.
- 1983            USDA Cash Award given by the BARC Area Director for contributions that helped to explain the role of antiozonant (EDU) and an enzyme (SOD) in the prevention of air pollution injury.
- 1985-87        Adj. Associate Professor of Agronomy, Univ. of Maryland.
- 1987            Research Excellence Award given by US-Environmental Protection Agency for work on National Crop Loss Assessment Network--A landmark study of air pollution effects on agriculture, at an International Air Pollution Meeting in Raleigh, N.C.
- 1987            Merit Pay Cash Award given by the BARC Area Director.
- 1988            Elected as Chairman of the 19th Taiwanese American Conference/East Coast, July 1-4, Penn State University, University Park, Pa.

Listed in American Men and Women of Science.

#### G. Other Significant Scientific Impact:

##### Presentation or Invited Seminars:

**Invited seminar:** February 10, 1986; "Chemical Modification of Environmentally Sensitive Plants " USDA/ARS, Frederick, MD.

**Invited seminar:** March 18, 1986; "Air Pollution and Plant Life". Washington Academy of Science, Georgetown University, DC.

**Presentation:** April 15-17, 1986; "Biochemical mechanism of ozone stress". Air Pollution Workshop Meeting, University of Maryland, College Park, Maryland.

**Presentation:** August 5, 1986; "Differential Aluminum Tolerances of Two Barley Cultivars related to organic acids in their roots". Intern'l Plant Nutrition Colloquium, USDA/ARS, Beltsville, MD.

**Invited Paper:** August 3-8, 1986; An in vitro system for studying the mechanisms of ozone tolerance and phytotoxicity in plant tissue culture system". International Congress of Plant Tissue and Culture, University of Minnesota, Minneapolis, Minn.



Presentation: August 6, 1986; "Effect of ozone on potato and tobacco suspension cell culture", at International Congress of Plant Tissue Culture, University of Minnesota, Minneapolis, Minn.

Presentation: June 8-13, 1986; "Rhythmic changes in ascorbic acid production in  $O_3$ -sensitive and tolerant soybean leaves in response to ozone stress". American Association of Plant Physiologist Society Annual Meeting, Baton Rouge, La.

**Invited Seminar:** April 4, 1987; "Environmental Problems in U.S. and effects of air pollutants on agricultural productivity". Nicholars Orem Middle School Advanced Biology Class, Hyattsville, MD.

Participated in International Conference on Tissue Culture Annual Meeting, May 27-28, 1987; at Crystal City, Washington, DC.

Presented two research papers at Joint PGRSA-JSCRP in Honolulu, Hawaii on August 2-August 6, 1987: 1) Effects of paclobutrazol on GA biosynthesis and fatty acid composition-- A case study on the differential sensitivity to  $SO_2$  stress in snapbean plants. 2) Efficiency of XE-1019 as a phytoprotectant against  $SO_2$  injury in snapbean.

**Invited paper presentation:** October 25-29, 1987; "Use of chlorophyll fluorescence as a diagnostic tool for differential tolerance of snapbean (*Phaseolus vulgaris* L.)". At International Conference on Assessment of Crop Loss from Air Pollutants. Raleigh, N.C.

Presentation: October 25-29, 1987; "Air Pollution, Plant, and Insect Interactions : Growth & Feeding Preferences of Mexican Bean Beetles on Bean Plants Stressed by  $SO_2$  and  $O_3$ ". At International Conference on Assessment of Crop Loss from Air Pollutants. Raleigh, N.C.

## VI. Publication :

### A). Peer Reviewed Publication:

1. Lee, E. H., J. K. Byun, and S. J. Wilding. 1985. A new gibberellin biosynthesis inhibitor, paclobutrazol (PP333), confers increased  $SO_2$  tolerance on snap bean plants. Envir. & Exp. Botany 25: 265-275.
2. Lee, E. H., J. K. Byun, and S. J. Wilding. 1985. Chemical modification of environmentally sensitive plants with GA biosynthesis inhibitors in response to  $SO_2$  stress. Proc. Plant Growth Reg. Soc. Amer. 12:152-58.
3. Lee, E. H., S. J. Wilding, K. Tuthill, M. Hartley, and D.



- Flanagan. 1986. An in vitro system for studying the mechanisms of  $O_3$ . VI. Int'l. Congress of Plant Tissue & Cell Culture. P. 453, Univ. of Minn., Aug. 3-8.
4. Lee, E. H. and C. D. Foy. 1986. Aluminum tolerance of two snap bean cultivars related to organic acids content evaluated by high-performance liquid chromatography. J. of Plant Nutrition 9: 1481-1498.
  5. Heggestad, H. E., T. J. Gish, E. H. Lee, J. H. Bennett, and L. W. Douglass. 1985. Interaction of soil moisture stress and ambient ozone on growth and yields of soybeans. Phytopathology 75: 474-477.
  6. Heggestad, H. E., J. H. Bennett, E. H. Lee, and L. W. Douglass. 1986. Effects of increasing of  $SO_2$  & ambient  $O_3$  on tomatoes: Plant growth, fruit yield and quality. Phytopathology 76:1338-44.
  7. Byun, Jae K., and E. H. Lee. 1986. The effect of paclobutrazol on the  $SO_2$  tolerance in apple seedlings. J. Korean Soc. Hort. 26:261-64.
  8. Lee, E. H., R. A. Saftner, S. J. Wilding, H. D. Clark & R. A. Rowland. 1987. Effects of paclobutrazol on GA Biosynthesis & Fatty acid composition. Proc. Plant Growth Reg. Soc. of Am. 14: 295-302.
  9. Lee, E. H. Use of chlorophyll fluorescence as a diagnostic tool for detecting differential tolerance of snapbean (P. vulgaris L.) to ozone stress. 1987. Environ. Pollu. .(accepted)
  10. Ku, J. H., D. T. Krizek, R. M. Mirecki & E. H. Lee. 1987. Influence of XE-1019 concentration on vegetative growth and  $SO_2$  sensitivity of P. vulgaris L. "Strike". Proc. Plant Growth Reg. Soc. Am. 14: 304-11.
  11. Heggestad, H. E., E. L. Anderson, T. J. Gish and E. H. Lee. 1988. Effects of ozone and soil water deficit on roots and shoots of field- grown soybeans. Environ. Pollution. (In Press)
  12. Mulchi, C. L., E. H. Lee, K. Tuthill, and E. V. Olinick. 1987. Influence of ozone stress on growth processes, yields and grain qual. character. among soybean cultivars. Environ. Pollution. (accepted).
  13. Foy, C. D. and Edward H. Lee. 1987. Differential aluminum tolerances of two barley cultivars related to organic acids in their roots. J. of Plant Nutrition. 10: 1089-1101.

C.) Book Chapters:

1. Bennett, L. H., E. H., E. H. Lee, and H. E. Heggestad. 1985.





Biochemical aspects of ozone and oxyradicals: Superoxide dismutase. A. J. Koziol and F. R. Whatley (eds.) Gaseous Air Pollutants and Plant Metabolism. Ch. 27, pp 413-424, Butterworths Press, London.

2. Lee, E. H., J. K. Byun and S. J. Wilding. 1987. Protection of crop plants from sulphur dioxide, chilling, and heat induced injury with paclobutrazol. In D.N. Rao et al., (ed.) Persp. in Environ. Botany 2: 147-61.

D.) Other Publications and Abstract:

1. Lee, E. H., J. K. Byun, and G. L. Steffens. 1985. Increased tolerance of plants to  $SO_2$ , chilling, and heat stress by a new GA biosynthesis inhibitor, paclobutrazol (PP333). Plant Physiol. 75: 66.
2. Bennett, J. H., E. H. Lee, and H. E. Heggestad. 1985. Physiological effects of  $SO_2$  and  $NO_2$ : Dose-Response Modeling. Plant Physiol. 77: 161.
3. Lee, E. H., J. K. Byun, and G. L. Steffens. 1985. A new gibberellin biosynthesis inhibitor, paclobutrazol (PP333). Plant Physiol. 77(S): 135.
4. Lee, E. H., J. K. Byun, S. J. Wilding, and G. L. Steffens. 1985. Paclobutrazol induced sulfur dioxide, chilling, and high temp. tolerance in snapbean plants. Proc. Plant Growth Reg. Soc. Amer. 12: 78.
5. Mulchi, C. L., W. J. Kenworthy, and E. H. Lee. 1985. Effects of  $O_3$  stress on N accumulation and relative growth rates for field-grown soybeans. Amer. Soc. of Agron. Proc. Agronomy Abstracts, p. 85.
6. Lee, E. H. 1986. Rhythmic changes in ascorbic acid production in  $O_3$ -sensitive and tolerant soybean leaves in response to  $O_3$  stress. Plant Physiol. 78: 16.
7. Zacharius, R. M., E. H. Lee, and S. J. Wilding. 1986. Effect of ozone on potato and tobacco suspension cell cultures. VI. Int'l. Congress of Plant Tissue and Cell Culture. p. 372. Univ. of Minn., Minneapolis, Minn., Aug 3-8.
8. Lee, E. H., Y. Wu and E. M. Barrows. 1987. Mechanistic studies of air pollution, interactions of Mexican Bean Beetles and bean plants. Plant Physiol. 83:73.
9. Millard, M.M., E. H. Lee and T. Krizek. 1987. Surface analysis and profiles of ions in plant leaves after exposure to gaseous air pollutants. Proc. Amer. Chem. Soc. (ASC Abstract).
10. Zacharius, R. M., J. A. Saunders and E. H. Lee. 1987. Method



for determining metabolite response of tobacco cell cultures to  $O_3$  stress. 27th Ann. Meetg. N. Am. Phytochem. Soc., Phytochem. Soc. 6/21-26, Univ. of Fl.



- I. Name: Robert M. Zacharius  
Title: Research Chemist  
Grade: GS-14
- II. CRIS Project No: 1209-23000-010  
Title: Biochemical Mechanisms of Tolerance to Air Pollutant and Environmental Stress in Bean and Tobacco Plants.
- III. Current Research Objectives and Progress (1987-1988) and Plans (1988):
- A. Objective: To ascertain if air pollution (in particular ozone) stress will induce tobacco cell cultures [derived from plants sensitive and plants tolerant to ozone ( $O_3$ )] to produce secondary ( $2^\circ$ ) metabolites (i.e., alkaloids and sesquiterpenes) and are their presence responsible for the observed tissue death from  $O_3$ .
- Found: In suspension cell experiments, exposure to  $O_3$  did not induce the accumulation of  $2^\circ$  stress compounds in either  $O_3$ -tolerant or  $O_3$ -sensitive cell lines. This fails to support contentions that phytotoxicity of de novo formed stress compounds are responsible for  $O_3$  damage to tobacco tissue.
- Plans: To repeat the above using leaves from  $O_3$  plants. To attempt to learn why the tobacco cell suspension fail to produce sesquiterpenoids or alkaloidal compounds when exposed to  $O_3$ . Are these cells incompetent to do so or is it a question of induction.
- B. Objective: To ascertain if  $O_3$ -stress of soybean cell suspensions would induce the formation of high levels of isoflavonoids or the de novo synthesis of isomeric pterocarpin glyceollins.
- Found: No major increase in the level of isoflavonoids was observed but a small amount of glyceollin was found in cultures exposed to high levels of  $O_3$ . Formation of glyceollin is probably a general stress response by soy cell suspensions and not unique to biotic induction.
- C. Objective: To ascertain if a specific protein(s)/enzyme(s) is associated with tobacco cell  $O_3$ -tolerance or  $O_3$ -sensitivity.
- Plans: (a) To examine protein/enzyme gel electrophoretic patterns of plant leaf blades and epicotyls of  $O_3$ -tolerant and  $O_3$ -sensitive tobacco plants and find changes which might occur in the patterns following exposure to  $O_3$  and/or free radical producing paraquat. Special attention will be given to isozymes of superoxide dismutase.
- (b) To examine the above in suspension cell culture derived from  $O_3$ -tolerant and  $O_3$ -sensitive plants and the changes which might occur following  $O_3$  and/or paraquat exposure.
- (c) Attempts will be made to isolate by column chromatography proteins implicated in the response in (a) and (b).



#### IV. Cooperators:

Dr. Kenneth Deahl, Vegetable Laboratory, ARS, Beltsville, MD.

Dr. James A. Saunders, Germplasm Quality and Enhancement Laboratory, ARS, Beltsville, MD.

Dr. John M. Ruth, Pesticide Degradation Laboratory, ARS, Beltsville, MD.

Dr. Charles Mischke, Weed Science Laboratory, ARS, Beltsville, MD

#### V. Curriculum vitae:

##### A. Educational History:

1943 - B.A. New York University: Major - Chemistry.

1948 - M.A. University of Colorado: Major - Organic biochemistry.

1953 - Ph.D. University of Rochester/Cornell Univ.: Major - Plant Physiology/Biochemistry.

##### B. Post-graduate Employment History:

1947-1948 - Graduate Teaching Assistant (chemistry), University of Colorado, Boulder, CO.

1948-1950 - Predoctoral Fellow (Nutrition Foundation), University of Rochester, Rochester, NY.

1950-1951 - Predoctoral Fellow (Nutrition Foundation) Cornell University, Ithaca, NY.

1951-1952 - Research assistant (Botany), Cornell University

1952-1954 - Research Chemist, General Cigar Co., Research Laboratory, Lancaster, PA.

1954-1984 - Research Chemist, GS-11, GS-12, GS-13, GS-14, USDA, ARS, Eastern Regional Laboratory, Wyndmoor, PA.

1967 Adjunct Lecturer (Biochemistry), Chemistry Department St. Joseph's College, Philadelphia, PA

1984-present - Research Chemist, GS-14, USDA, ARS, Beltsville, MD

##### C. Society Offices Held:

Chemical Society of Washington - Program Committee, 1986-87.

Potato Association of America - Potato Physiology Committee, 1975-77

##### Membership in Professional Societies:

American Chemical Society, 1948 - present

American Society of Plant Physiologists, 1952 - present.

The Biochemical Society (U.K.) 1964 - present.

American Phytopathological Society - 1974 - present

International Association for Plant Tissue Culture - 1977-present

Phytochemical Society of North America - 1985-present

National capital Area Branch Tissue Culture Association - 1986 - present

Sigma Xi - 1947 - present





Editorial Board Appointments:

None

E. Advisory and Consultant Appointments (since 1980):

1. Plant Tissue Culture Panel for AID/SCI 1986.  
 Panel Chairman of Plant Tissue Culture Panel for AID/SCI 1987.  
 Appointed by the chairman to help organize a Tissue Culture Symposium for ATTC (Rockville, MD) for Oct. 1987.  
 Member of the Eastern regional Research Center Library Committee, 1974-1983.  
 Served on various committees at Fairs examining Science projects (High School and Jr. High School) for Montgomery County, PA, held at Ursinus College from 1970-1984. Often chaired such committees.  
 Reviews manuscripts for botanical and biochemical journals.
2. General Consulting Activities:  
 Consulted by Dr. G. Maclachlan, McGill University, Montreal (1981) on the organization of The Plant Biotechnology Workshop at the ASPP-CSPP joint meeting, Laval University.  
 Was one of four speakers opening the Workshop.
3. ARS Activities:

G. Other Significant Scientific Impact:

Invited seminars: (since 1981):

Invited to present a seminar at the Rohm and Haas Research Center, Springhouse, PA, Oct. 21, 1982. Seminar title: Stress Metabolite Induction in Plants.

Invited by Dr. P. Kadkade to present a seminar at GTE Laboratories, Life Sciences Department, Waltham, MA, Oct. 1983.

Invited to present seminar at ARS, BARC, April 1984.  
 Title: Stress Metabolites in Potatoes and Soybean Cell Suspension Cultures.

Invited to present seminar at ARS, Kearneysville, WVA, April, 1984.

Invited to present seminar at ARS, Frederick, MD, Apr. 1984.

Invited to deliver seminar on phytoalexins at Biotechnica International (date to be set), Spring 1988.

Visiting Scientist at Dr. Oluf Gamborg's Plant Cell Culture Laboratory, National Research Council of Canada, Saskatoon, Sask., Canada, Mar.-June 1977.



Was informed by the Institute for Scientific Information that the publication by Zacharius, Zell, Morrison and Woodlock, Anal. Biochem. 30:148-152(1969) was cited in the literature often enough to be regarded as a Citation Classic. 1981.

A USDA coop. student working under the incumbent's direction received the Exxon Student Paper Award at the Nat'l Organization for the Professional Advancement of Black Chemists and Engineers, held in New York City, May 5-8, 1982. Charles Abney, a student from Lincoln University presented: Isoflavonoids and glyceollins in stressed suspension cultures.

Invited to present seminar at the Plant Tissue Culture Group, ARS, Beltsville, March 1985.

Invited to present a seminar at a proposed ATTC Symposium in Oct. 1987 on phytoalexins in plant tissue culture. Conference was cancelled.

#### Publications:

Since 1985:

01. Zacharius, R. M., Kalan, E. G. and Kimoto, W. I. (1985). Biotransformation of potato stress metabolites, rishitin, lubimin and 15-dihydrolubimin by potato and soybean cell suspension cultures. Plant Cell Reports 4:1-3.

#### Other Publications and Abstracts:

01. Zacharius, R. M., Lee, E. H., and Wilding S. 1986. Effect of ozone on potato and soybean suspension cell cultures. Proc. 6th Internat'l congress of Plant Tissue and Cell Culture, University of Minnesota, Minneapolis.
02. Zacharius, R. M. 1986. Problems with the use of plant cell suspension cultures for biochemical/physiological/pathological studies. 26th Annual Meeting of the Phytochemical Society of N.A. July 13-17. Univ. of Maryland, College Park.
03. Zacharius, R. M., Saunders, J. A. and Lee, E. H. 1987. Method for Determining Response of Tobacco Cell Cultures to Ozone Stress. 27th Annual Meeting of the Phytochemical Society of N.A. June 21-26, University of South Florida, Tampa.



I. Name: Howard E. Heggstad

Title: "Retired" Research Plant Pathologist

II. Recent Award: Received Award for Research Excellence 1980-1984, a recognition TO USDA AGRICULTURAL RESEARCH SERVICE, BELTSVILLE by scientists Howard Heggstad, Jesse Bennett, Edward Lee, the THE ENVIRONMENTAL PROTECTION AGENCY, the period covers the years we participated in the National Crop Loss Assessment Network.

III. Scientific Activities:

February 5-7, 1985. Member of Peer Review Panel, Agricultural Effects Research National Acid Precipitation Program, at Brookhaven National Laboratory, Brookhaven, New York.

February 3-5, 1986. Invited Participant in National Council Air & Stream Improvement Forestry Workshop, sponsored by federal, state and private agencies, at Atlanta, Georgia.

September 17, 1986. Invited by scientists of the U.S. Geological Survey to examine along with Drs. Wergin, Millard, Fleming and Lee, plant specimens from the Lake Nyos Gas Disaster in Cameroon, West Africa. Contribution cited in Science 236:169-175. "The Lake Nyos Gas Disaster in Cameroon, West Africa", by Kling, G. W., et al. 1987.

September 30, 1986. Testimony concerning the effects of ozone on crop commodities, before the Committee on Public Works, U.S. Senate, Hearings on S. 2203, "The New Clean Air Act."

March 4-12, 1987. Member of Peer Review Panel, Terrestrial Effects Research, National Acid Precipitation Program at Atlanta, Georgia.

October 25-29, 1987. Co-Chairman of Session III "Yield Assessment using Field Approaches for Measuring Crop Loss" in the International Conference: Assessment of Crop Loss from Air Pollutants, at Raleigh, North Carolina. Session Chairman at the conference and handled editorial review of papers before and after the conference. Invited papers will be published in book and contributed papers in a special issue of Environmental Pollution. Both publications by Elsevier Applied Science Publishers Ltd., England.

January 20, 1988. Invited Participant in EDU Workshop at University of Massachusetts, Amherst, Mass.

April 10-14, 1988. Special Invitee to Twentieth Annual Air Pollution Workshop at Penn. State, University Park, PA. This is a special 20th Anniversary Meeting of the Air Pollution Workshop.



#### IV. Publications:

1. Heggstad, H. E. 1986. Effects of air pollutants on pome fruits. In Virus and Virus Like Diseases of Pome Fruits. USDA Handbook. P.R. Fridlund Ed., Washington State Univ., Prosser VA. Manuscript accepted for publication.
2. Filmore, M. I., Thomas C. A., and Heggstad, H. E. 1986. Effect of the pollutant ozone in ambient air on lime beans. *J. Agric. and Food Chem.* 34:179-185.
3. Heggstad, H. E., Bennett, J. H., Lee, E. H., and Douglass, L. W. 1986. Effects of increasing doses of sulfur dioxide and ambient ozone on tomatoes: plant growth, leaf injury, elemental composition, fruit yields and quality. *Phytopathology.* 76:k338-1344.
4. Heggstad, H. E., Anderson, E. L., Gish, T. J., and Lee, E. H. 1988. Effects of ozone and soil water deficit on roots and shoots of field-grown soybean. *Environ. Pollution* 50: (In press, accepted 4 Aug. 1987).
5. Heggstad, H. E. 1988. Reduction in soybean seed yields by ozone air pollution? *J. Air Pollut. Control Assoc.* Submitted March 1988.
6. Heagle, A. S., Kress, L. W., Temple, P. J., Kohut, R. J., Miller, J. E. and Heggstad, H. E. 1988. Factors influencing ozone dose-yield response relationships in open-top chamber studies. In Assessment of crop loss from air pollutants. Proceedings of International conference, Raleigh, N.C., USA, ed. by W. W. Heck, O. C. Taylor, and D. T. Tingey, London, Elsevier Applied Science.
7. Bennett, J. H., Lee, E. H., and Heggstad, H. E. 1988. Inhibition of photosynthesis and stomatal conductance interactions induced by SO<sub>2</sub>, NO<sub>2</sub> and SO<sub>2</sub>+NO<sub>2</sub>: stress response modeling. *Atmos. Environ.*
8. Heggstad, H. E., Lee, E. H., and Gish, T. J. In preparation. Effects of ambient ozone and soil moisture stress on root and shoot development and yields of soybeans in soil columns.







I. Name Donald T. Krizek  
 Title Plant Physiologist  
 Grade GM-15

II. CRIS Project No.: 1209-23000-007

Title: Role of light, temperature and water stress on the chloroplast antioxidant system.

III. Technical Assistance:

Roman M. Mirecki, GS-9, Plant Physiologist, M.S. Botany (Support Scientist).

Steven P. Dubik, GS-5, Biological Lab Technician  
 (Vacant at present)

Post-Doctorate, GS-11/12, Plant Physiologist/Chemist  
 (Candidate currently being recruited).

IV. Current Research Objectives and Progress

A. Objective: Determine the physiological and morphogenetic mechanisms of adaptation to root restriction in tomato.

Progress: Root restriction caused a marked reduction in growth of the lateral buds in soybean, tomato and euonymus, suggesting a possible impairment in the synthesis or transport of cytokinins from the root system. Uptake of several critical elements including Ca and P was also impaired. Studies conducted using reciprocal grafts between dwarf and normal tomatoes demonstrated that cytokinin-like activity was under genetic control and that the scion determined the extent of root growth (In collaboration with S. Dubik, M. S. Ruff, R. M. Mirecki, D. W. Inouye, A. Carmi, F. W. Snyder, J. A. Bunce, J. van Staden and C. Forsyth).

Plans: A post-doctoral research associateship was awarded for a proposal to investigate the "Mechanisms of plant adaptation to water stress and root restriction." A Plant Physiologist/Chemist, GS 11/12 is being recruited for this position. Tomato plants will be subjected to root restriction in a specially designed membrane system and exudate collected for analyses. Specific objectives will be to determine: (1) the role of abscisic acid (ABA) and cytokinins in altering cell membrane permeability and tissue sensitivity; (2) the role of organic and inorganic solutes in osmotic adjustment; and (3) the influence of genotype and root morphology on the uptake and/or transport of P, Ca, and K in root-restricted plants in relation to composition, ion distribution and ultrastructure of the root.

B. Objective: Determine the physiological and ultrastructural effects of Al toxicity x water deficit x genotype interactions in tomato.

Progress: Aluminum toxicity and water deficit had a synergistic effect on the growth of Al-tolerant and Al-sensitive cultivars of



barley, sunflower, and tomato grown in an acid, Al-toxic Tatum subsoil under controlled environmental conditions. Under high Al stress and high water stress, chloroplasts from the Al-sensitive sunflower cultivar 'Romania HS-52' were smaller and contained less starch than chloroplasts from the Al-tolerant cultivar 'Manchurian'. The smaller chloroplasts tended to have fewer grana stacks per unit area than did the chloroplasts from tolerant plants. These differences were not apparent when the Al-sensitive cultivar was grown either in the absence of Al or water stress. (In collaboration with C. D. Foy, W. P. Wergin, S.N.K. Rao, R. M. Mirecki, and C. Coradetti).

Plans: Continue collaborative studies with Dr. Foy and Dr. Wergin to elucidate the role of drought in the acid soil complex using tomato genotypes that differ in root hair density and efficiency in P uptake.

- C. Objective: Determine hormonal and biochemical mechanisms involved in phytoprotection against SO<sub>2</sub> injury in coleus by temperature pretreatment at 13°C.

Progress: After 5 days of temperature pretreatment at 13°C, both SO<sub>2</sub>-sensitive ('Buckley Supreme') and SO<sub>2</sub>-insensitive ('Marty') cultivars of coleus had significantly greater concentrations of ABA in their leaves and one third to one half the stomatal conductance and transpiration rate as those kept at 20°C for 5 days. Correspondingly, plants hardened at 13°C showed protection against SO<sub>2</sub> injury while those maintained at 20°C were severely damaged. (In collaboration with Paul Terry, Helen Norman, Roman Mirecki, Peter Semeniuk, Edward Lee).

Plans: Since 13°C is close to the critical temperature for phase change in membrane properties, these studies are being expanded to investigate possible changes in lipid metabolism in hardened coleus and tomato plants in collaboration with Dr. Helen Norman and Dr. Judy St. John. Correlative measurements will be made of changes following temperature pretreatment in endogenous levels of carbohydrates and in the activity of key enzymes and metabolites involved in scavenging free radicals and preventing lipid peroxidation (e.g., ascorbic acid, carotenoids, glutathione reductase, ascorbate peroxidase, catalase). Plans are also being made to look at changes in EPR signals in leaves of hardened and non-hardened plants of tomato and coleus in conjunction with Dr. Charles Caldwell. Cooperative studies are also underway with Dr. Paul Terry to develop an assay for ABA in guard cells using a monoclonal antibody obtained from Dr. Steven Quarrie, in England.

- D. Objective: Determine the mechanism by which triazoles ameliorate SO<sub>2</sub> and chilling injury and prevent photoinhibition under high levels of photosynthetic photon flux.



Progress: Dose-response studies were conducted to determine the efficacy of using uniconazole as a phytoprotectant against SO<sub>2</sub> injury in snap bean 'Strike' and 'Eagle'. This compound differs from paclobutrazol by only a single double bond, but was found to be 2 to 10 times more effective than paclobutrazol in providing protection against SO<sub>2</sub> exposure in coleus and bean. Uniconazole treatment applied as a soil drench 13 days prior to SO<sub>2</sub> fumigation had little or no effect on stomatal conductance but reduced transpiration in snap bean on a whole plant basis by nearly 40%. Since the maximum inhibition of the rate of stem elongation and leaf enlargement required at least 0.1 to 0.5 mg pot<sup>-1</sup> of uniconazole, while protection was obtained at 0.02 mg pot<sup>-1</sup>, it appears that the phytoprotective effects of this compound in bean are not necessarily related to its growth-retarding properties as an anti-gibberellin. (In collaboration with Dr. Ja Hyeong Ku, visiting scientist from Chungnam National University, Korea, Roman Mirecki, Dr. Edward Lee).

Plans: The possible role of triazoles in scavenging free radicals, in altering endogenous levels of ethylene and polyamines, and in increasing water use efficiency will be investigated in chilling-sensitive and chilling-tolerant cultivars under low and high PPF levels, using selected inhibitors of ethylene and polyamine biosynthesis.

#### V. Cooperators:

Plant Stress Laboratory: William Wergin, Charles Foy, Al Fleming, Edward Lee, Charles Caldwell, Merle Millard.

Plant Hormone Laboratory: Paul Terry, Autar Matoo, George Buta, Werner Meudt.

Florist and Nursery Crops Lab: Peter Semeniuk, Mark Roh, John Neal, Robert Griesbach.

Weed Science Laboratory: Helen Norman, Judy St. John.

Horticultural Crops Quality Laboratory: Judy Abbott, Harold Moline, Chien Yi Wang.

Instrumentation Research Laboratory: Karl Norris, David Massie.

Fruit Laboratory: George Steffens

Vegetable Laboratory: Tom Barksdale

University of Maryland: Stephen P. Dubik, Doris Rodriguez, James Healy, David Hershey.

University of Wisconsin: Ted Tibbitts, Dennis Stimart.

National Bureau of Standards: Donald McSparron.





National Aeronautics and Space Administration: John Sager,  
Ralph Prince, Thomas Dreschel.

University of Natal, UN/CSIR Research Unit for Plant Growth and  
Development, Department of Botany, Pietermaritzburg, 3200 Republic  
of South Africa: J. van Staden

Indian Institute of Horticultural Research, Bangalore, India: S.K.  
Tikoo, Srinivasa N.K. Rao.

Chungnam National University, Department of Horticulture, College  
of Agriculture, Daejeon, South Korea: Ja Hyeong Ku.

The Volcani Research Center, Institute of Soils and Water, P O Box  
6, Bet Dagan, Israel: Avner Carmi

## Curriculum Vitae:

### A. Educational History:

- 1957 - B.A., cum laude, Adelbert College, Western Reserve  
University, Cleveland, Ohio; major, botany; minor,  
chemistry, math.
- 1958 - M.S., University of Chicago, major, botany.
- 1964 - Ph.D. University of Chicago, major, botany (plant  
physiology).

### B. Professional Positions:

- 1958-1962 Scientist Special (Biologist), U.S. Air Force, Arctic  
Desert, Tropic Information Center, Research Studies  
Institute, Maxwell AFB, Montgomery, Alabama.
- 1959-1961 Instructor in Biology (Part-time), Montgomery Center,  
University of Alabama, Montgomery, Alabama.
- 1964-1966 Instructor, Botany and Biology, The University of  
Chicago, Chicago, Illinois.
- 1966-1972 GS-12/13, Plant Physiologist, USDA, ARS,  
Phyto-Engineering Laboratory, Beltsville, MD.
- 1972-1988 GS-13/14, Plant Physiologist, USDA, ARS, Beltsville,  
Plant Stress Laboratory, Maryland.
- 1988- GM-14/15, Plant Physiologist, USDA, ARS, Plant Stress  
Laboratory, Natural Resources Institute, Beltsville,  
Maryland.

### C. Society Offices Held:

- American Society of Plant Physiologists:
  - Secretary Treasurer, Washington Section, 1970-71
  - Vice Chairman, Washington Section, 1971-72
  - Chairman, Washington Section, 1972-73
  - Member of Career Brochure Committee, 1980-82





American Society for Horticultural Science (ASHS):

ASHS Working Group on Controlled Environments and Growth Chambers:

Co-founder and member, 1969-

Chairman, 1974-76

ASHS Working Group on Developmental and Environmental Stress Physiology:

Member, 1976-

Chairman, Water Stress Subgroup, 1981-82

Chairman, 1982-84

North Central Regional (NRC-101) Committee on Growth Chamber Use:

ARS Representative, 1974-

Secretary, 1978-79

Vice Chairman, 1979-80

Chairman, 1980-82

American Society of Agricultural Engineers:

Member, Plant and Animal Physiology Advisory Committee, 1969-

Representative to the American Society for Heating, Refrigeration, and Engineering (ASHRAE) Liaison (SE-303) Committee, 1972-

White House Task Force on Inadvertent Modification of the Stratosphere:

USDA Representative, 1975-78

Chairman, Subcommittee on Biological and Climatic Effects Research, 1975-78

EPA Interagency Task Group on Biological and Climatic Effects Research:

Chairman, 1977-80

D. Membership in Professional Societies:

Foreign

British Society for Experimental Biology, 1966-

Commission Internationale De L'Eclairage (CIE), United States

National Committee, Division 6. Photobiology and Photochemistry, 1988-

International Society for Horticultural Science, 1968-

Japanese Society of Plant Physiologists, 1964-

International Plant Propagators Society, 1971-76

Scandinavian Society for Plant Physiology, 1963-

American

American Association for the Advancement of Science, 1959-

American Institute of Biological Sciences, 1957-

American Polar Society, 1957-

American Society for Horticultural Science, 1966-

American Society of Plant Physiologists, 1957-

Southern Section, 1960-

Midwestern Section, 1964-

Washington Section, 1966-

Botanical Society of America, 1957-

Botanical Society of Washington, 1967-

Council for Optical Radiation Measurements, 1984-

Mid Atlantic Society of Molecular Biology, 1984-

National Association of Biology Teachers, 1960-72



New York Academy of Sciences, 1974-  
 American Society for Photobiology, 1974-  
 Phytochemical Society of North America, 1986-  
 Plant Growth Regulator Society of America, 1976-  
 Tissue Culture Society of America, Wash. D.C. Section, 1984-

E. Editorial Board Appointments:

Member, Editorial Board, Scientia Horticulturae, 1984-86  
 Member, Editorial Board, Phytotronics, Japanese Journal of  
 Environmental Control, 1987-

F. Advisory and Consultant Appointments:

1. Advisory:

Graduate advisor to Mr. Stephen P. Dubik, M.S. student,  
 Department of Horticulture, University of Maryland

Sponsor and technical advisor to Ms. Miriam Ruff, Senior Honors  
 Project, Department of Zoology, University of Maryland,  
 1985-1987.

Hosted visiting scientist from Korea, Dr. Ja Hyeong Ku, who  
 spent a year in my laboratory on a Korean National Science  
 Foundation Grant. Sept. 1986 - Aug. 1987.

Hosted visiting scientist from India, Dr. S. N. K. Rao, FAO  
 Fellow, Indian Institute of Horticultural Research, Bangalore,  
 India. Sept. - Dec. 1986.

Served as sponsor for Science Fair projects for two seventh  
 grade high school students from Virginia, Ms. Leslie Gillum and  
 Ms. Tamara Wexler. Leslie's project on UV-Radiation Effects on  
 Cucumber won first prize in the National Science Fair and a free  
 one week trip to attend the International Science Fair in Puerto  
 Rico. Tamara's project on Gamma Radiation Effects in Tomato  
 also took top honors. Jan. - June 1987.

Reviewed numerous manuscripts for various journals including  
 Plant Physiology, Journal of the American Society for  
 Horticultural Science, HortScience, Crop Science, Agronomy  
 Journal, Botanical Gazette, American Journal of Botany,  
 Physiologia Plantarum, Environmental and Experimental Botany.

Reviewed research proposals for various organizations including  
 National Science Foundation, BARD, AID, NASA, and AIBS.

2. General Consulting Activities:

National Academy of Sciences, Committee on Impacts of  
 Stratospheric Change and Panel on Atmospheric Chemistry,  
 1975-



National Aeronautics and Space Administration/American Institute  
Biological Sciences/Controlled Ecological Life Support  
System (NASA/AIBS/CELSS)  
AIBS Plant Physiology Advisory Panel, 1979-  
Chairman, NASA/AIBS/KSC Biomass Production Technical Panel,  
1987-

Commission International De L'Eclairage (Commission on  
Illuminating Engineering). Division 6. Photobiology and  
Photochemistry, United States National Committee, 1988-

### 3. ARS and BARC Activities:

Served as ARS representative at the Department of Energy Seminar  
on Carbon Dioxide Research, held at the National Academy of  
Sciences, at the request of Dr. Doral Kemper, NPS, Dec. 3-4,  
1986.

At the request of Dr. Tom Army, ARS Associate Administrator,  
provided input for the President's speech in Canada on the  
effects of increased UV-B radiation, July 1987.

At the request of Dr. Doral Kemper, NPS, and Dr. Walter Heck, NC  
State University, assisted in development of USDA position  
statement submitted to OMB concerning federal research needed to  
assess the impact of stratospheric ozone reduction, Oct. 1987.

Briefed Dr. Waldemar Klassen, Area Director, BARC, and Dr. Lee  
Comer, detailed to the Secretary's Office on the stratospheric  
ozone issue, Dec. 1987.

Appointed USDA Representative to the Interagency Committee for  
Stratospheric Ozone Protection, April 1, 1988, by Assistant  
Secretary, Science and Education, Dr. Orville G. Bentley.

### G. Honors, Grants and Awards:

- a. J. Paul Visscher Award of the Cleveland Audubon Society, 1953.
- b. Cleveland Press Scholarship, Western Reserve Univ., 1953-54.
- c. Adelbert College Scholarships, Western Reserve University,  
1954-57.
- d. Scholarship Loan, Federation of Women's Clubs of Greater  
Cleveland, for 1955.
- e. Beta Beta Beta, 1955.
- f. Karl Lemmerman Prize for outstanding research paper, 1955.
- g. Ralph Allen Spengler, Jr. Award for excellence in botany, 1957.
- h. Wychwood Fellowship in Botany, University of Chicago, for  
1957-58.
- i. Hutchinson Fellowship in Botany, University of Chicago, 1958.
- j. Sigma Xi, 1958.
- k. Leaders in American Science, 1959.
- l. American Men and Women of Science, 1960.
- m. NSF Fellowship in Botany, University of Chicago, for 1962-64.
- n. British Society for Experimental Biology, 1966.
- o. Who's Who in the East, 1970.
- p. NSF Grants for conducting Baseline Growth Studies, for 1973-78.  
Co-Principal Investigator.





- q. Department of Transportation Grant for Climatic Impact Assessment Program for 1972-75. Co-Principal Investigator.
- r. Contingency Funds from ARS Administrator for UV-B Research for 1975-76. Principal Investigator.
- s. Environmental Protection Agency Grant for Biological and Climatic Effects Research (BACER) Program. Co-Principal Investigator, 1976-78.
- t. NSF travel grant to attend the International Workshop on Effects of Ultraviolet Radiation on Plants, New Delhi, India, November 1-5, 1982. Also received funds from USDA/FERRO and AID.
- u. Men of Achievement, 1988.
- v. Awarded a cash award for research on plant adaptation to environmental stress, 1988.
- w. Awarded ARS Research Associateship for proposal entitled: Physiological mechanisms of plant adaptation to water stress and root restriction", by the ARS Administrator Dr. Terry Kinney, Jr., 1988.

#### H. Other Significant Scientific Impact:

##### 1. Invited Seminars:

- a. Invited to lecture on "Accelerated Culture Under Controlled Environments", and "Plant Response to Environmental Stress", in the Peoples Republic of China, as member of Horticultural Delegation, U.S. - China Science Exchange Program, July 13-31, 1985.
- b. Invited to present seminar entitled "Plant Adaptation to Environmental Stress: Physiological and Morphological Mechanisms", Department of Horticulture, University of Maryland, April 18, 1988.

##### 2. Invited Symposia:

- a. Invited to present paper at the International Society of Arboriculture meeting in San Antonio, Texas, on "The Influence of Water Stress and Restricted Root Volume on the Growth and Development of Urban Trees", August 12, 1986.
- b. Invited to present paper on "Abiotic Factors Affecting Crop Loss Assessment", at the International Conference on "Assessment of Crop Loss from Air Pollutants", Raleigh, North Carolina, October 25-29, 1987.
- c. Invited to participate in NATO Workshop on "Biochemical and Physiological Mechanisms Associated With Environmental Stress Tolerance in Plants", University of East Anglia, Norwich, England, August 2-7, 1987.
- d. Invited to participate in British Society for Experimental Biology Session on "Plants Under Stress: Biochemistry, Physiology and Ecology and Their Application to Plant Improvement", University of Lancaster, England, March 28-31, 1988.





- e. Invited to participate in National Academy of Sciences/National Research Council Workshop on Ultraviolet Radiation, April 28-29, 1988.

### 3. Other Invitations:

- a. Selected as Chairman, Session on Environmental Stress Physiology, XXII International Horticultural Congress, University of California, Davis, CA, August 15, 1986.
- b. Invited to edit a series of volumes for CRC Press on Photobiology of Bacteria, Fungi and Plants; Soilless Culture; Measuring and Reporting Environmental Conditions in Controlled Environment Chamber Research.
- c. Invited to write four chapters for the Growth Chamber Manual to be published by Academic Press. 1987. (Manuscripts in review or in preparation).

## I. Publications:

### A. Peer Reviewed Publications:

1. Krizek, D. T., A. Carmi, R. Mirecki, F. W. Snyder, and J. A. Bunce. 1985. Comparative effects of soil moisture stress and restricted root zone volume on morphogenetic and physiological responses of soybean [Glycine max (L.) Merr.]. J. Expt. Bot. 36(162):26-38.
2. Krizek, D. T., P. Semeniuk, H. E. Moline, R. M. Mirecki, and J. A. Abbott. 1985. Chilling injury in coleus as influenced by photosynthetically active radiation, temperature, and abscisic acid pretreatment. I. Morphological and physiological responses. Plant, Cell & Environ. 8:135-142.
3. Krizek, D. T., W. P. Wergin, and P. Semeniuk. 1985. Morphological and physiological properties of poinsettia leaves and bracts in relation to sulfur dioxide sensitivity. Env. Exp. Bot. 25(2):165-173.
4. Krizek, D. T. 1985. Methods of inducing water stress in plants. Proc. ASHS Symposium on Water Stress Measurement Techniques - Instrumentation and Procedures. HortScience 20(6):1028-1038. 1985. (Invited Review; Peer Reviewed Society Proceedings)
5. Krizek, D. T., R. M. Mirecki, and P. Semeniuk. 1986. Influence of soil moisture stress and abscisic acid pretreatment in modifying SO<sub>2</sub> sensitivity in poinsettia. J. Amer. Soc. Hort. Sci. 111(3):446-450.



6. Tibbitts, T. W., D. A. McSparron, and D. T. Krizek. 1986. Spectral effects on the use of photon flux sensors for measurement of photosynthetic photon flux in controlled environments. *Biotronics* 15:31-36.
7. Abbott, J. A., D. T. Krizek, P. Semeniuk, H. E. Moline, and R. M. Mirecki. 1987. Refreshed delayed light emission and fluorescence for detecting pretreatment effects on chilling injury in coleus. *J. Amer. Soc. Hort. Sci.* 112(3):560-565.
8. Krizek, D. T. and S. P. Dubik. 1987. Influence of water stress and restricted root zone volume on growth and development of urban trees. *J. Arboric.* 13(2):47-55.
9. Ruff, M. S., D. T. Krizek, R. M. Mirecki, and D. W. Inouye. 1987. Restricted root zone volume: Influence on growth and development of tomato. *J. Amer. Soc. Hort. Sci.* 112(5):763-769.
10. van Staden, J., A. Carmi, C. Forsyth, and D. T. Krizek. 1987. Cytokinin-like activity in the roots and shoots of tomatoes following reciprocal grafts between normal and dwarf genotypes. *S. Afr. J. Bot.* 53(4):276-278.
11. Fleming, A., D. T. Krizek, and R. M. Mirecki. 1987. Influence of ammonium nutrition on the growth and mineral composition of two chrysanthemum cultivars differing in drought tolerance. *J. Plant Nutr.* 10:1869-1881.
12. Krizek, D. T., R. M. Mirecki, A. L. Fleming, and S. P. Dubik. 1987. LPS-induced chlorosis in chrysanthemum as influenced by genotype and ammonium/nitrate ratio. *J. Plant Nutr.* 10:1059-1069.
13. Terry, P. H., D. T. Krizek, and R. M. Mirecki. 1988. Genotypic variation in coleus in the ability to accumulate abscisic acid in response to water deficit. *Physiol. Plant.* In press.
14. Krizek, D. T. and C. D. Foy. 1988. Role of water stress in differential aluminum tolerance of two barley cultivars grown in an acid soil. *J. Plant Nutr.* In press.
15. Krizek, D. T. and C. D. Foy. 1988. Mineral element concentration of two barley cultivars in relation to water deficit and aluminum toxicity. *J. Plant Nutr.* In press.
16. Krizek, D. T., C. D. Foy, and W. P. Wergin. 1988. Role of water stress in differential aluminum tolerance of six sunflower cultivars grown in an acid soil. *J. Plant Nutr.* In press.



17. Krizek, D. T. and C. D. Foy. 1988. Mineral element concentration of six sunflower cultivars in relation to water deficit and aluminum toxicity. J. Plant Nutr. In press.
18. Britz, S. P. and D. T. Krizek. 1988. Irradiance regimes for a controlled ecological life support system (CELSS). In: Physiological and Engineering Considerations for Design of a Controlled Ecological Life Support System. AIBS/KSC Biomass Production Technical Panel Report. American Institute of Biological Sciences, Washington, D.C. In press.

B. Book Chapters:

1. Krizek, D. T. 1986. Photosynthesis, dry matter production and growth in CO<sub>2</sub> enriched atmospheres. Chapter 17. pp. 193-225. In: J. R. Mauney and J. M. Stewart (eds.), Cotton physiology. Reference Book Series No. 1. The Cotton Foundation, Memphis, TN.

C. Other Publications and Abstracts:

1. Krizek, D. T., A. Carmi, R. Mirecki, F. W. Snyder, and J. A. Bunce. 1985. Comparative morphogenetic and physiological effects of soil moisture stress and restricted root zone volume on soybean. Plant Physiol. Suppl. 77(4):94. 1985. (Abstract)
2. Krizek, D. T., P. Semeniuk, and R. M. Mirecki. 1985. Influence of soil moisture stress and abscisic acid pretreatment in modifying SO<sub>2</sub> sensitivity in poinsettia. HortScience 20(3):533. 1985. (Abstract)
3. Krizek, D. T., P. Semeniuk, and R. M. Mirecki. 1985. Influence of temperature and PAR pretreatment in modifying SO<sub>2</sub> sensitivity in poinsettia. HortScience 20(3):603. 1985. (Abstract)
4. Dubik, S. P., D. P. Stimart, and D. T. Krizek. 1985. Influence of restricted root zone volume on vegetative growth and partitioning of assimilate in spreading euonymus. HortScience 20(3):584. (Abstract)
5. Terry, P. H., D. T. Krizek, and R. M. Mirecki. 1985. Determination of endogenous abscisic acid in plant leaves using HPLC and GC. 190th American Chemical Society Meeting, Chicago, IL. (Abstract)
6. Terry, P. H., D. T. Krizek, and R. M. Mirecki. 1985. Correlations between abscisic acid levels and plant water status measurements in leaves of coleus plants under soil moisture stress. Proc. Plant Growth Reg. Soc. Amer. 12:188-193. 1985. (Society Proceedings)





7. Krizek, D. T., R. M. Mirecki, and P. Semeniuk. 1986. Influence of paclobutrazol concentration and time of pretreatment in ameliorating SO<sub>2</sub> injury in coleus. *Plant Physiol. Suppl.* 80(4):125. (Abstract)
8. Tikoo, S. K., D. T. Krizek, R. M. Mirecki, S. P. Dubik, and T. H. Barksdale. 1986. Response of ten tomato genotypes to water deficits. *HortScience* 21(3):815. (Abstract)
9. Krizek, D. T., P. Semeniuk, and R. M. Mirecki. 1986. Influence of temperature and PPF pretreatment in modifying SO<sub>2</sub> sensitivity in coleus. *HortScience* 21(3):816. (Abstract)
10. Krizek, D. T., P. Semeniuk, R. M. Mirecki, and G. L. Steffens. 1986. Influence of triazoles in ameliorating SO<sub>2</sub> injury in coleus. Abstracts, Phytochemical Soc. North Amer.
11. Terry, P. H., D. T. Krizek, and R. M. Mirecki. 1986. Influence of photosynthetic photon flux, spectral quality, and temperature on chlorophyll and abscisic acid concentrations in leaves of coleus plants. *Proc. Plant Growth Reg. Soc. Amer.* 13:84-90. (Society Proceedings)
12. Krizek, D. T., A. Fleming, R. M. Mirecki, and S. P. Dubik. 1986. Growth and development, chlorophyll content, and mineral composition of tomato as influenced by ammonium and nitrate nutrition and exposure to water stress. Abstracts Internat. Plant Nutr. Colloq.
13. Millard, M. M., E. H. Lee, and D. T. Krizek. 1987. Surface analysis and profiles of ions in plant leaves after exposure to gaseous air pollutants. *Amer. Chem. Soc. Abstracts.*
14. Dubik, S. P., D. T. Krizek, and D. P. Stimart. 1987. Growth analysis of Euonymus kiautschovica Loes. 'Sieboldiana' plants grown in large and small root zone volumes. *HortScience*. 22(5):1092. (Abstract)
15. Ruff, M. S., D. T. Krizek, R. M. Mirecki, and D. W. Inouye. 1987. Restricted root zone volume: Influence on growth and development of tomato. *HortScience*. 22(5):1081. (Abstract)
16. Krizek, D. T., R. M. Mirecki, and P. Semeniuk. 1987. Influence of paclobutrazol pretreatment and SO<sub>2</sub> dose rate on SO<sub>2</sub> sensitivity and transpiration rate in coleus. *HortScience*. 22(5):1155. (Abstract)
17. Ku, J. H., D. T. Krizek, R. M. Mirecki, and E. H. Lee. 1987. Efficacy of XE-1019 as a phytoprotectant against SO<sub>2</sub> injury in snap bean. *Proc. Plant Growth Reg. Soc. Amer.* 14:304-311. (Conference Proceedings)





18. Krizek, D. T., C. D. Foy, S. N. K. Rao, C. Coradetti, and R. M. Mirecki. 1988. The role of water stress in differential aluminum tolerance of two tomato cultivars differing in drought tolerance. Abstract for annual meeting of the Amer. Soc. Plant Physiol., Reno, Nevada, July 10-14, 1988. I.



- I. Name : Robert Howell  
 Title: Research Plant Pathologist  
 Grade: GM-13
- II. CRIS Project No: 1209-23000-009  
 Title: Selection of Stress Resistant Rhizobium and Legume Gene Sources to Increase N<sub>2</sub>-Fixation
- III. Current Research Objectives and Progress (1986-1987) and Plans (1988)
- A. Objective: Determine if a relationship between tolerance of higher temperatures and drought tolerance exists.
- Progress: Two hundred sources of peanut germplasm have been grown for 5 days at either 27, 32, 37 or 42 C. Growth of radicals and the ratios of seed dry weights to seed dry weights have been determined. One hundred and 50 of these lines were planted in the field and were evaluated for drought tolerance on 7/30, 8/7, 8/13 and 8/21 during the droughty season of 1987.
- Plan: Statistically analyze our data to evaluate the premise of the objective and continue to search for drought tolerance techniques and drought tolerant germplasm.
- B. Objective: Identify the influence of Bradyrhizobium strains on mineral content of peanut and soybean tissues.
- Progress: Cultivars of both peanuts and soybeans are grown in the presence of individual Bradyrhizobium strains. Plants are separated into parts and analyzed for several minerals. Strains influence the mineral content of peanut tissues. Soybean tissues await analyses.
- Plan: Analyze soybean tissues and evaluate the possible use of Bradyrhizobium strains to improve the nutritional balance in plants.
- C. Objective: Identify the influences of tillage systems on mineral content of peanuts.
- Progress: Three peanut cultivars are grown in each of three tillage systems 1) conventional plow, 2) in-row plant, and 3) band-till at Suffolk, Va. in 1985, 1986 and 1987. Plants from each treatment were collected on 5 dates during each year, separated into several components, and prepared for analyses.
- Plan: Complete the tissue mineral analyses and consider experiments to determine the influence of tillage systems on Bradyrhizobium strains and their influence on nitrogen fixation.



#### IV. Cooperators:

Dr. Terry Coffelt, Peanut Geneticist, Tidewater Research Center, USDA, ARS-VPI, Suffolk, VA.

Drs. Scott Wright, Agricultural Engineer, and Morris Porter, Plant Pathologist, Tidewater Research Center, USDA, ARS-VPI, Suffolk, VA.

Dr. George Buta, Research Chemist, Plant Hormone Lab, ARS, Beltsville, MD.

Dr. William P. Wergin, Plant Physiologist, Plant Stress Lab, ARS, Beltsville, MD.

Dr. Claude McKee and Mr. R. Mulford, Agronomists, Department of Agronomy, University of Maryland, College Park, MD

Dr. R. C. Leffel, Research Geneticist, Soybean Genetics and Nitrogen Fixation Lab, ARS, Beltsville, MD

Dr. John Teasdale, Plant Physiologist, Weed Science Lab, ARS, Beltsville, MD

#### V. Curriculum Vitae:

##### A. Educational History

1959, B.S. University of Md. Agronomy

1961, M.S. " " Agronomy

1964, Ph.D. " " " Plant Pathology-Nematology

##### B. Post-Graduate Employment History

1964-1967 Research Associate, Department of Botany, University of Maryland, College Park.

1967-present ARS, Research Plant Pathologist, Beltsville, MD.

##### C. Society Offices Held:

Member of Finance Committee, American Peanut Research and Education Society.

##### Membership in Professional Societies:

Sigma Xi

Alpha Zeta

American Society of Agronomy

American Phytopathological Society

American Peanut Research Education Society

##### D. Editorial Board Appointments:

Participate as ad-hoc reviewer for manuscripts for Peanut Science, the Agronomy Journal and Plant Diseases.



E. Advisory Appointments:

Graduate Student Advisor for Kay Calavan M.S., Agronomy Department,  
University of Maryland

Special Advisor, Eleanor Roosevelt High School for Senior Research  
Project of Elizabeth Allgood.

F. Awards:

None

G. Publications:

1. Coffelt, T. A. and R. K. Howell. 1986. Effect of ethrel seed  
treatment on growth, yield, and grade of two Virginia-type peanut  
cultivars. Peanut Sci:13:60-63.

2. Howell, R. K. 1987. Rhizobium induced mineral uptake in peanut  
tissues. J. Plant Nutrition 10:1297-1305.

H. Abstracts:

1. Howell, R. K. and J. G. Buta. 1985. MH-30, BCC3, and Bud-Nip: their  
influence peanut seed yields and grade characteristics. Proceed.  
APRES.37.
2. Howell, R. K. and T. A. Coffelt. 1986. Rhizobium: their effect on  
mineral uptake in peanut foliage. Agron Abstracts:114.
3. Howell, R. K., F. S. Wright, and D. M. Porter. 1987. Tillage  
systems: their influence on nutrient content of peanut tissues.  
Proceed. APRES.27.





- I. Name: Charles R. Caldwell  
Title: Plant Physiologist  
Grade: GM-13  
Technical Assistance: Michael McMahon (Biol. Lab. Tech., GS-7)
- II. CRIS Project No.: 1209-20172-011-00D  
Title: Biophysical and biochemical properties of membranes in plant responses to environmental stress.
- III. Current Research Objectives and Progress (1985-87) and Plans (1988)

- A. Objective: Characterize temperature-induced changes in plant plasma membrane lipid composition and structure.

Progress: Growth of wheat and barley seedlings at 4 °C results in rapid changes in root plasma membrane lipid composition. The amount of phospholipid unsaturated fatty acid increases. The level of stigmasterol decreases with increases in campesterol and sitosterol. Electron paramagnetic resonance spectroscopy (EPR) of spin-labeled membranes isolated from wheat seedlings grown at 20 and 4 °C indicated that these lipid changes resulted in a more "fluid" membrane at 4 °C. The "fluidity" of the lipids at 4 °C of membranes from 4 °C-grown plants is the same as that of the 20 °C-grown plants at 20 °C. These results suggest that the temperature-induced changes in lipid composition are an adaptive response of the the plants to maintain the normal biophysical and biochemical processes of the membranes at reduced growth temperature.

Plans: A more detailed time-dependence will be performed for the temperature-induced changes in wheat root plasma membrane phospholipid fatty acid and free sterol composition. Also, the temperature-dependence of these lipid changes will be examined. New phospholipid and sterol spin labels are currently being synthesized to permit a detailed EPR investigation of the membrane structural changes induced by variations in sample temperature and lipid composition.

- B. Objective: Characterize temperature-induced changes in plant plasma membrane protein composition and structure.

Progress: EPR and fluorescence spectroscopy have been utilized to measure temperature-induced changes in the protein dynamics of plasma membranes isolated from the roots of barley and wheat seedlings grown at 20 °C. Intrinsic protein fluorescence and the rates of protein tryptophan photoionization indicated alterations in membrane protein conformation between 12 and 32 °C. EPR of covalently spin-labeled membrane proteins also demonstrated a temperature-dependent change in protein mobility at 12 °C. Lipid spin probes were used to show changes in lipid lateral diffusion rates above 12 °C. These results suggest the presence of lipid microstructures at physiological



temperatures which may influence the activity of membrane-bound enzymes.

Plans: Most of the research described above had been performed on the root plasma membrane of 20 °C-grown barley seedlings. Considering the results described in Objective A, a study will be performed on the temperature-induced changes in membrane protein dynamics of plasma membranes isolated from wheat seedlings grown under differing temperature conditions. This will permit the characterization of the role of membrane lipid composition in the control of membrane protein conformation.

- C. Objective. Isolate and characterize plasma membrane proteins which bind heavy metal cations.

Progress. The binding of various metal cations to high affinity sites on barley and wheat root plasma membrane proteins has been investigated by protein-enhanced terbium fluorescence. Wheat root plasma membrane proteins which bind Al(III), Ni(II), Cu(II), Cd(II) and Zn(II) have been isolated by immobilized metal affinity chromatography (IMAC). Electrophoretic analyses of these proteins suggest that a protein of 80 kd can associate with a variety of polyvalent cations. Fifty six and 76 kd proteins may selectively bind aluminum.

Plans: High gradient magnetic filtration and magnetic field flow fractionation will be evaluated for use in the isolation of solubilized wheat root plasma membrane proteins which have bound ferro- or paramagnetic cations (e.g. Fe(II,III), Mn(II), Tb(III), Gd(III)). These methods should be more selective than IMAC. An affinity chromatography support with an immobilized organomercury compound has been prepared recently to isolate cysteine-rich proteins from the wheat seedlings. Since such cation-binding proteins as phytochelatin and calmodulin are cysteine-rich, the proteins isolated with this material may bind metal cations. The metal-protein coordination will be studied by EPR.

- D. Objective: Determine the role of plasma membrane phosphoinositides in stress-induced signal transduction in plants.

Progress: Since this is a new objective, progress has been limited to the synthesis of a series of novel nitrophenol derivatives of phospholipid polar head groups. These compounds will permit the chromogenic assay of phosphatidylinositol-dependent phospholipase C (PIPlase C).

Plans: PIPlase C has recently been found in plant plasma membrane preparations. Using the chromogenic substrates mentioned above, a simple assay can be performed to verify the presence of this enzyme in wheat root plasma membranes. Assuming this enzyme is present, the temperature- and metal



cation-dependences of the enzyme activity will be determined.

- E. Objective: Purchase and install a Bruker ESP-300 electron paramagnetic resonance spectrometer.

Progress: Contingency funds were made available by the Area Office at the end of FY 1986 to purchase an EPR. A laboratory was renovated at little expense to ARS in the spring of 1987 to house the instrument. After a 13 month adventure with procurement, the instrument arrived in December, 1987. The required cooling system arrived in February, 1988. The EPR is now fully functional and is being utilized as described above and in other cooperative research.

Plans: Objective completed.

#### IV. Cooperators (Current and Anticipated):

Plant Stress Laboratory: M. Millard, D. Krizek, E. Lee, A. Fleming and C. Foy.

Hormone Laboratory: A. Matoo and F. Calahan.

Weed Science Laboratory: H. Norman and J. St. John.

#### V. Curriculum Vitae:

##### A. Educational History:

1973-B.S. Tulane University; major, biology  
1975-M.S. Tulane University; major, biology  
1981-Ph.D. Michigan State University; major, botany

##### B. Post-graduate Employment History:

1981-1985 GS-12, Plant Physiologist, USDA, ARS, BARC  
1985-pres. GM-13, Plant Physiologist, USDA, ARS, BARC

##### C. Member of Professional Societies:

American Society of Plant Physiologists  
Biophysical Society  
Scandinavian Society for Plant Physiology

##### D. Editorial Board Appointments:

none

##### E. Advisory and Consultant Appointments and Other Activities:

Reviewed numerous grant applications for USDA, NASA, NSF and BARD.





Reviewed manuscripts for Plant Physiol., Physiol. Plant. and Planta.

#### F. Honors and Awards:

Awarded ARS postdoctoral research associate for "Investgations of the role of the plasma membrane in the stress-induced responses of cell sultures derived from cereals of differing tolerances to environmental stress and their genetic variants", by Dr. Terry Kinney, Jr., Administrator of ARS, 1984.

Awarded USDA Certificate of Merit with cash bonus "For the acquisition of surplus properties that have expanded several laboratory capabilities without requiring additional expense to the laboratories or BARC", 1984.

Awarded postdoctoral research associateship by the DOE Plant Research Laboratory, Michigan State University, to continue an investigation of the role of the plasma membrane in plant responses to environmental stress, 1981.

Awarded graduate research assistantships by the DOE Plant Research Laboratory, Michigan State University, to conduct biochemical and biophysical studies of plant plasma membranes under environmental stress, 1975-1981.

Awarded honors in biology by Tulane University Department of biology for B.S. research, 1973.

#### G. Other Significant Scientific Impact:

Invited to present an "Epitome of Emerging Technology" on electron paramagnetic resonance to the BARC Area Office, 1986.

Invited to edit a book on plant biomembranes by MacMillan publishers, 1987.

#### I. Publications:

##### A. Peer Reviewed Publications:

Caldwell, CR and CE Whitman 1987 Temperature-induced protein conformational changes in barley root plasma membrane-enriched microsomes. I. Effect of temperature on membrane protein and lipid mobility. Plant Physiol 84: 918-923.

Caldwell, CR 1987 Temperature-induced protein conformational changes in barley root plasma membrane-enriched microsomes. II. Intrinsic protein fluorescence. Plant Physiol. 84: 924-929.





B. Book Chapters:

Haug, AR and CR Caldwell 1985 Aluminum toxicity in Plants: The role of the root plasma membrane and calmodulin. pp. 359-381, Frontiers of Membrane Research in Agriculture, JB St. John, E Berlin and PC Jackson, eds. Rowman and Allanheld, Totowa.

C. Other Publications and Abstracts

Caldwell CR 1985 An electron paramagnetic resonance study of temperature-induced changes in barley root membrane-bound proteins. Plant Physiol 77(S): 147 (abstract).

Whitman CE and CR Caldwell 1986 Characterization of hexokinase from wheat roots and its inhibition by aluminum. Plant Physiol 80(S): 121 (abstract).

Caldwell CR 1986 Immobilized metal affinity chromatography of barley root plasma membrane-bound proteins. Plant Physiol. 80(S): 80 (abstract).

Whitman CE and CR Caldwell Characterization of hexokinase from wheat roots and its inhibition by aluminum. Submitted to Plant physiol.



- I. Name : Merle M. Millard  
Title: Research Chemist  
Grade: GM-14
- II. CRIS Project No: 1209-23000-019  
Title: Analysis of Environmental Stress at Plant Leaf and Root Surfaces by X-Ray Photoelectron Spectroscopy
- III. Current Research Objectives and Progress (1986-87) and Plans (1988)
- A. Objective: Investigate the surface and interface chemistry in the interaction of gaseous air pollutants with plant leaves.
- Progress: Initial study of leaf surface changes after SO<sub>2</sub> and ozone fumigation of plants completed.
- Plans: Investigate leaf surface changes resulting from the interaction of NO<sub>2</sub> with plants.
- B. Objective: Investigate the surface and interface chemistry in the interaction of metals and nutrients with plant root and leaf interfaces.
- Progress: Surface compositional and structural changes in barley roots after exposure to aluminum were analyzed by XPS. A protocol to prepare root segments for analysis by XPS was developed.
- Plans: Investigate effects of pH, nutrient ion composition, and complexes capable of complexing aluminum on the surface of barley roots. Investigate the adsorption, translocation and uptake of metal and nutrient ions at plant leaf and root interfaces by XPS.
- C. Objective: Investigate the adsorption and translocation of herbicides through the cuticle region of plant leaves and roots.
- Plans: The interaction of selected herbicides containing elements with higher XPS detection sensitivities, i.e., fluorine with leaf surfaces and the effect of environmental factors including light on this process will be investigated.
- IV. Cooperators:
1. E. H. Lee, C. D. Foy, C. R. Caldwell, W. P. Wergin, Plant Stress Laboratory, ARS, Beltsville, MD
  2. C. F. Mischke, Weed Science Laboratory, ARS, Beltsville, MD



V. Curriculum Vitae:

A. Educational History:

- 1958-B.S. University of California, Berkeley, major Chemistry
- 1963-Ph.D. Purdue University, West Lafayette, Indiana, major Inorganic Chemistry.

B. Post-graduate Employment History:

- 1963-1964 Instructor, Purdue University, IR-Raman Spectroscopy of Metal Carbonyls
- 1964-1968 Asst. Professor, University of Iowa, Iowa City, Iowa, Inorganic chemistry, organometallic silicon chemistry
- 1968-1969 Research Manager, Tracerlab, 2030 Wright Ave., Richmond, CA, Excited Gas Technology
- 1969-1970 Vice President, Plasmatics, 2400 Wright Ave., Richmond, CA, Excited Gas Technology
- 1970 Visiting Scientist, Inorganic Materials Research Division, Lawrence Radiation Lab., Univ. of Calif., Berkeley, CA, X-ray photoelectron spectroscopy
- 1970-1979 Research Scientist, Fiber Science, USDA, ARS, Western Regional Research Laboratory, 800 Buchanan St., Albany, Calif.
- 1979-1980 Visiting Scientist, Plasma and Thin Film Dept., IBM Research lab., 5600 Cottle Rd., San Jose, CA 95193
- 1980-1984 Research Chemist, USDA, ARS, Western Regional Research Lab., Albany, CA.
- 1984-present Research Chemist, Plant Stress Laboratory, USDA, ARS, Beltsville, MD

C. Society Offices Held:

None

Member in Professional Societies:

American Society of Plant Physiologists

American Chemical Society

D. Editorial Board Appointments:

None



# E. Professional Advisory and Consultant Appointments:

## Technical Advisory Activities:

- 1971      Trapelo West, Division of LFE Corp., 1601 Trapelo Road, Waltham, Massachusetts Consultant for Trapelo on contract between Trapelo and Falcon Plastics, Los Angeles. Development of plasma process instrument for treatment of polystyrene plastic dishes for attachment of tissue cells.
  
- 1972      Department of the Navy, Mare Island Naval Shipyard, Vallejo, California. Investigation of flow discharge process for applying fluorocarbon coatings to glass surface. Process for applying fluorocarbon coatings to glass surface. Project #773532. Agreement #2 6N00221-2. Funded for \$22,500.
  
- 1973      Department of the Navy, Mare Island Naval Shipyard, Vallejo, California. Deposition of fluorocarbon polymer coatings by gas phase ultraviolet irradiation of fluorocarbon monomers. Project #774452001. Agreement #2 6N00221-2. Funded for \$22,500.
  
- 1974      Southern Regional Research Laboratory, New Orleans, Louisiana. Consulted with Dr. Soignet and others on experience at WRRRC with surface analysis by XPS. Dr. Soignet previously consulted with Dr. Millard concerning purchase of XPS instruments at WRRRC.
  
- 1975      University of Utah, Department of Materials Science. Consulted with Dr. Andrate and others concerning the use of XPS to investigate biological materials. WRRRC has had considerable experience in this area and our experience with biological sample preparation and data handling were of interest to his group. A seminar was presented during the visit to the Materials Science Department entitled "Application of XPS to the Study of Biological Surfaces."
  
- 1975      NASA AMES Research Center Biotechnology Division, Moffett Field, CA. Performed XPS surface analysis for T. Wyden van and A. T. Bell on reverse osmosis membrane project. A. T. Bell, T. Wyden van and C. C. Johnson. J. Appl. Polym. Sci. 19, 1911-30 (1975).
  
- 1976      University of California, San Francisco, Department of Endocrinology. Consulted with Dr. L. Pickart on the surface analysis of hepatoma cells. Surface and calcium depth profile analysis on cells at WRRRC resulted in the location of calcium in the cell membrane during pyruvate induced DNA synthesis.





- 1976 WRRC. M.S. Masri and V.G. Randall consulted on metal ion binding determination using XPS. Proc. 1st Int. Conf. on Chitin.Chitosan, 1978. R.A.A. Muzzarelli ed. MT SEA Grant Report MIT SG 78-7, #78307, pp 277-87.
- 1977 Department of Chemical Engineers, UCB. Collaborated with A.T. Bell, M. Shen and K. NaKajima on XPS analysis of fluorocarbon films.
- 1977-78 Department of Chemical Engineers, UCB. Collaborated with R. W. Benedict and M. C. Williams on discharge treatment of surfaces for erythrocyte adhesion to plastic. Biomat., Med. Dev., Art. Org. 7(4):457-93. 1979.
- 1979 Continuing project with UC SF Dept. of Endocrinology.
- 1979 Continuing project with UCB Engr. Dept.
- 1981 Pub. #55. Collaboration with D. L. Perry, Materials Science, LBL, XPS Analysis of solid uranyl hydrolysis products.
- 1986 Collaboration with Dr. W. T. Anderson, NRL, Washington, D.C. XPS Analysis of irradiation damage to group 3/5 devices.

F. Honors and Awards:

- 1963 Union Carbide Fellow, Purdue University
- 1965 Research Corporation Research Grant for Beginning Scientists, \$6,000.00 Research on Organometallics.
- 1965-68 Department of the Army, Durham, \$40,000.00 research on Organometallics.
- 1972-74 Mare Island Naval Shipyard Interscience Support Agreement, \$32,500.00. "Radiofrequency Glow Discharge and UV Photopolymerization Deposition of Fluorocarbon Films on Glass."
- 1975 Younger Textile Scientist Award. Expenses for travel and participation in 5th International Wool Conference Aschen, Germany and week tour of German Industry. Awarded by the German Government.
- 1979-80 IBM Fellowship Program Visiting Scientist, Research Department, San Jose, CA. Expenses for one year.
- 1983 ARS Research Associate Award, "Isolation of Plasma Membrane Proteins from Plant Protoplasts" \$40,000.00, 2 year Post Doctorial and \$10,000.00 for supplies.
- 1986 Naval Research Laboratory Interagency Service Agreement "GaAs" Radiation effects study by XPS, \$15,000.00.



G. Special Invitations:

- (1) Invited to contribute article in Textile Research Journal on Surface Analysis of Fibers, by the editor. 1972.
- (2) Invited to contribute chapter entitled, "Synthesis of Organic Polymer Films in Plasmas," in book Techniques and Applications of Plasma Chemistry, John R. Hollahan, ed., John Wiley & Sons, New York, 1974.
- (3) Invited to contribute paper entitled "Surface Analysis of Plasma-Polymerized Fluorocarbon Films by XPS," in symposium on Plasma Chemistry of Polymers at 169th ACS Meeting, Philadelphia, Pennsylvania, 1975. Resulting paper published in Journal and book.
- (4) Invited to participate and present paper entitled "Applications of ESCA to Surface Analysis of Textile and Polymers," at Physical electronics Industries (PHI) sponsored seminar "Trends in Application of Electron Spectroscopy to Materials," Edina, Minnesota, 1975. PHI is a leading manufacture of electron spectrometer instruments.
- (5) Invited to present a paper entitled "Surface and Depth Profile Analysis of Tissue Culture Cells and Bacterial via XPS and Oxygen Plasma Etching," in symposium on Spectroscopy of surfaces, Pacific Conference on Chemistry and Spectroscopy, Los Angeles, California.
- (6) Invited to participate and present paper entitled "Surface Analysis of Wool Fibers and Polymers by XPS," in symposium Advances in Characterization of Metal and Polymer Surfaces, Division of Organic Coatings and Plastics Chemistry, ACS Meeting, New York, 1976.
- (7) Invited to present paper entitled "Surface and In-Depth Characterization of Fibers and Biological Cells Using ESCA and Oxygen Plasma Etching," at 28th Annual Summer Symposium on Analytical Chemistry, Arizona State University, June 1976.
- (8) Invited to contribute review article to Advances series entitled "Contemporary Topics in Analytical and Clinical Chemistry" by series editor, D. M. Hercules, 1977.
- (9) Expenses paid for two day visit to Physical Electronics Division of Perkin Elmer Eden Prairie, Minnesota, a manufacturer of XPS instrumentation. Presented a seminar on XPS Analysis of biological Samples and escape Depths through Monolayers. 4/27/79.
- (10) Surface Analysis of films and Escape Depth Through Monolayers Seminar at IBM Seminar at IBM Research, San Jose, Thin Film and Plasma Department. 4/2/79.
- (11) Surface Analysis by XPS. Seminar at Chevron Research, Richmond, CA 10/25/79.



- (12) Invited by symposium chairman to contribute paper at symposium on Reactions at Fiber Surface. Cellulose Paper and Textile Division, 180th ACS Meeting, Las Vega, Nevada, Aug. 1980.
- (13) Invited speaker in the Symposium of Industrial Applications of Surface Analysis, L. A. Casper and C. J. Powell, Chairmen and the International Symposium on Physicalchemical Aspects of Polymer Surfaces K. L. Mittal, Chairman, both at the 191st meeting of the American Chemical Society, New York, Aug. 1981.
- (14) Invited to organize and chair a Symposium on Surface Chemistry for one day at the Pacific Conference on Chemistry and Spectroscopy, Oct. 27-29, 1982, Cathedral Hill Hotel, San Francisco, Calif. by the program committee.
- (15) Invited to present a lecture in the Symposia on Recent Advances in Surface and Film Analysis, by F. M. Mirabella, Symposia Chairman at the Federation of analytical chemistry and Spectroscopy Societies FACSS II, Sept. 16-21 Marriott Hotel, Philadelphia, PA. 1984
- (16) Invited to lecture in the Symposium on the Surface Characterization on Fibers at the Eastern Electron Spectroscopy Society (EESS), 1985 Fall Meeting Union Carbide Corp., Bound Brook, NY by Dr. Everhard, Symposium Chairman.
- (17) Invited speaker at the Eastern Electron Spectroscopy Society Spring Meeting, Goodstay Center, Wilmington, DE, May 19, 1987.
- (18) Invited speaker in the Symposium on Polymer Surface Science, J. F. Rabolt and J. M. Pochan organizers in Division of Polymer Chemistry 194th American Chemistry Society meeting, Aug. 30-Sept. 4, 1987, New Orleans, LA.

## VI. Publications:

### A. Peer Reviewed Publications:

Sandeliu, A.S., Penel, C. Auderset, G., Brightman, A., Millard, M.M. and Morre, D.J. "Isolation of Highly Purified Fractions of Plasma Membrane and Tonoplast from the Same Homogenate of Plant Cells by Free-Flow Electrophoresis. Plant Physiol. 81:177-185. 1986.

### B. Other Publications and Abstracts:

Millard, M. M., Mattingly, B. L. and Altman, S. A., "Bilayer Disposition of Protoplast Plasma Membrane Proteins." Plant Physiol. 77, p. 71 (Abstract) 1985.

Millard, M. M., Mattingly, B. L. and Altman, S. A., "Surface Exposed Rye Protoplasts Plasma Membrane Glycoproteins." Plant Physiol. 80, p. 94 (Abs. #497). 1986.

Millard, M. M. Surface Analysis and Profiles of Ions in Plant Leaves after Exposure to Gaseous Air Pollutants. (Abstract) Abstracts of the 194th National American Chemical Society Meeting Aug. 30-Sept 4. New Orleans, LA 1987.





- I. Name : Eugene L. Vigil  
 Title: Research Plant Physiologist/Cell Biologist  
 Grade: New Hire  
 Technical Assistance:  
     L C Frazier (Biol. Lab. Tech., GS-9)  
 Partial assistance:  
     Eric Erbe (Biol. Lab. Tech., GS-9)  
     Norita Chaney (Biol. Lab. Tech., GS-9)  
     Christopher Pooley (Photographic and Computer Specialist  
     - EM Lab.)
- II. CRIS Project No.: 1209-23000-008-0D  
 Title: Biophysical and biochemical properties of membranes  
         in plant responses to environmental stress
- III. Current Research Objectives and Progress (1985-87) and Plans (1988)
- A. Overall Objective: The long range objective is to target plants of specific genetic potential and response to environmental stress for attacking the global problem of producing oil seeds of high quality. The main focus is membranous organelles involved in cellular processes critical to food storage, germination and seedling growth. Key organelles, whose development and function play a major and vital role in determining seed quality, are being evaluated. Protein bodies have been identified as the cellular organelles directly affected by environmental stress caused by whole plant defoliation.
- B. Objective: Establish whether or not the plasma membrane in cotton seeds is an intact bilayer structure. This information was vital to clarify long standing confusion over the site of mineral and small molecule leakage from seeds during imbibition.
- Progress: In the absence of any reliable method for examining the fine structure of dry seed tissue, an extensive empirical series of investigations was made to develop and analyze new methods. The result was introduction of anhydrous and combined anhydrous-hydrous methods for obtaining reliable and reproducible data on the fine structure of membranes in dry seeds. This was complemented by introduction of a new method of using unfixed tissue for preparation of freeze-fracture replicas without cryoprotection. This application was introduced after analysis of moisture content in the cotyledon and radicle revealed that there was generally 12% moisture in these major seed organs. The amount of ice crystal formation under this condition of low moisture was minimal. The results from different experimental approaches was identical, namely that the plasma membrane and membranes generally were intact bilayers having essentially the same architecture of membranes in fully hydrated tissue.





Plans: Objective completed.

- C. Objective: Establish whether food stored in radicles was used in germination of seeds and whether application of environmental stress affected the process.

Progress: The presence of large amounts of protein within cortical cells of cotton radicles provided the basis for pursuing experiments to determine the significance of this observation in relation to seed quality and sensitivity to environmental stress. The results from this study established that up to the time of germination (radicle protrusion) seeds were not adversely affected. Once the radicle emerged, drying caused major damage to the plasma membrane and remaining lipid bodies within cortical cells. At the time of radicle emergence, there was little protein left in the cells, presumably having been used during earlier stages of imbibition. These observations may provide a basis for establishing strategies to deal with reduced plant stands caused by drought or chilling.

Plans: Objective essentially completed except for possible extensions in future experiments on seed germination with seeds from stressed plants.

- D. Objective: Develop a simple reproducible procedure to test the impact of environmental stress on food storage in cotton radicles during seed development.

Progress: After testing the impact of a chemical spray to defoliate cotton plants containing numerous bolls at different stages of maturation and obtaining equivocal results, the design was changed. The original hypothesis that whole plant defoliation was a good model for studying the impact of simulated natural environmental stress, e.g. drought or nutrition, but the experimental procedure needed modification. Whole plant defoliation was found to provide a simple reproducible method for effecting nutritional and drought stress on developing seeds.

Plans: Use experimental procedure in future field and green house experiments to obtain samples for biochemical and fine structural analyses. These experiments are designed to establish additional data on the time-course of stress effects and substantiate further the impact on protein body development. The pattern of protein synthesis and protein complement of whole radicles and isolated protein bodies (where possible) will be pursued. These experiments and those indicated below represent a program for an ARS competitive grant proposal which went to panel on April 6th.



- E. Objective: Analyze seeds from stress experiment to determine germinability and cellular sites of nutritional and drought stress.

Progress: A causal relationship was found between the duration of stress received during seed ripening and per cent germination. An in vitro test was developed for excised radicles to demonstrate that radicle elongation during germination was solely dependent on food stored therein. Having confirmed this point, the fine structure of the cortex was examined. Previous biochemical and fine structural research showed that this tissue contains large stores of lipid and protein in lipid and protein bodies, respectively. Radicles from seeds which had received 30 days of nutritional and drought stress contained less protein and fewer protein bodies which consisted mainly of large vacuoles with small regions of compact protein. Fine structural examination of tissue sampled at ten day intervals from 30 days after anthesis to boll opening at 60 days after anthesis revealed that there was impairment of vacuole partitioning to form the protein body population common to control seeds. The amount of protein present in vacuoles/protein bodies was also reduced in proportion to the amount of stress the seed received. These results are important to the seed industry for determining quality and germinability.

Plans: Continue analysis of impact of nutritional and drought stress on protein body development through random sampling and processing microscopic data by image analysis with the Cambridge Quantimet 970 system. Continue collaboration with Dr. Rob Donaldson on further characterization of protein profile with SDS - PAGE for radicles from control and stressed seeds. If the pending proposal with the ARS competitive grants office is approved for funding, support is requested for a biochemist - research associate. Dr. Tung Kwang Fang who has worked with us previously has been identified as that person.

#### IV. Cooperators:

- Dr. William P. Wergin, Mr. L C Frazier, Mr. Eric F. Erbe, Mrs. N. Chaney, Mr. Christopher Pooley, Plant Stress Laboratory, ARS, Beltsville, MD.
- Dr. Robert Yaklich, Germplasm Quality Enhancement Laboratory, ARS, Beltsville, MD
- Dr. Robert Donaldson, Biology Department, George Washington University, Washington, D.C.



V. Curriculum Vitae:

A. Educational History:

1959 - B. S. Loyola University, Chicago; Major - Biology

1963 - M. S. University of Iowa, Iowa City; Major - Botany  
(Plant Physiology)

1967 - Ph.D. University of Iowa, Iowa City; Major - Botany  
(Plant Physiology)

B. Post Graduate Employment History:

1967-69 - NIH Postdoctoral Fellow, Botany, University of Wisconsin,  
Madison, WI.

1969-71 - PHS Postdoctoral Fellow, Biology, University of Chicago,  
Chicago, IL.

1971-79 - Asst. Prof. Biology, Marquette University, Milwaukee, WI.

1979-81 - Asst. Prof. Botany, University of Maryland, College Park,  
MD.

1981-present - Research Associate, Horticulture, University of  
Maryland, College Park, MD.

C. Society Offices Held:

President - Milwaukee Horticultural Society, 1978-79

Program Chairman - Histochemical Society, 1976-79

Councilor - Histochemical Society, 1979-82

Membership in Professional Societies:

American Society of Cell Biologists

American Society of Plant Physiologists (Washington Chapter)

Electron Microscopic Society of America (Chesapeake Chapter)

German Histochemical Society

Histochemical Society

New York Academy of Sciences



Fellow of Royal Microscopic Society

Society for Advancement of Chicanos and Native Americans in Science

D. Editorial Board Appointments: None

E. Advisory and Consultant Appointments:

1. Advisory: None

2. General Consulting Activities:

Member of Cell Biology Panel for National Science Foundation, 1984-87.

Ad hoc reviewer for National Science Foundation:

- 1). Cell, Molecular and Developmental Biology Section - 1981 - present;
- 2). Minority Research Centers of Excellence (MRCE) Program: 1988 -

3. ARS and BARC Activities - New Hire

F. Awards:

Elected Fellow of Royal Microscopic Society - April, 1987

G. Other Significant Scientific Impact:

Accepted invitation to make presentation and write chapter on "Ultrastructural Histochemistry with High Voltage Electron Microscopy (HVEM) for American Zoological Society Symposium, Philadelphia, PA (1985) Expenses paid.

Accepted invitation to make presentation and write chapter on "Structural Changes in Protein Bodies of Cotton Radicles during Seed Maturation for International Symposium on Botanical Microscopy, York, England (1985) Expenses paid.

Accepted invitation to make presentation on "Hypertension" which was recorded and published as a chapter in a book Searching, Teaching, Healing: American Indians and Alaskan Natives in Biomedical Careers under the sponsorship of the National Institutes of Health (1986) Expenses paid.

Accepted invitation to prepare chapter on "Isolation of Glyoxysomes" for volume 148 of Methods in Enzymology (1987).

Invited to be organizer of minisymposia for botanical histochemistry for International Congress of Histochemistry and Cytochemistry in Washington, D.C., August, 1988 (1987).







Accepted invitation to be minisymposium speaker on  
"Quantitative Image Analysis of Cell Organelles" at  
International Congress of Histochemistry and  
Cytochemistry, Washington, D.C., August, 1988.

Accepted invitation to be co-editor for special issue of  
International Cell Biology Reports covering the  
minisymposium on botanical histochemistry at the  
International Congress of Histochemistry and  
Cytochemistry, Washington, D.C., August, 1988.

#### VI. Research Needs:

- A. Office and Laboratory: As a new hire, I need designated office and laboratory space. The research program I have developed has reached a stage where a wet laboratory is essential. In addition, the large amount of electron microscopy I do for my research requires having space in the EM facility for myself and my assistant. My pending proposal with ARS competitive grants office, if funded, provides support for a research associate/biochemist. This person will require office space and access to a wet laboratory. To meet these conditions and provide close interaction with personnel for efficient performance of experiments, I need an office in close proximity to a designated laboratory.
- B. Personnel: I presently have one full time bio. tech. and depend on three other bio. tech. on a part time basis. Their assistance is vital to continued progress of my research program.
- C. Laboratory Equipment: The research program I have developed has progressed to the point where application of biochemical methodologies is essential to continued progress. The laboratory requested above will need major equipment in addition to basic laboratory supplies, especially an electrophoresis system, centrifuge and ancillary equipment for organelle isolation and characterization through enzyme and protein analysis. My pending grant proposal has only a modest budget for equipment, mainly the electrophoresis system. While sharing centrifuges is acceptable, there is a clear need for a quality dual beam spectrophotometer with temperature control around the cuvettes.

#### VII. Publications:

##### A. Peer Reviewed Publications (1985-88)

1. Vigil, E. L., R. L. Steere, W. P. Wergin and M. N. Christiansen. 1985. Structure of plasma membrane in radicles from cotton seeds. *Protoplasma* 129:168-177.



2. Vigil, E. L., and M. Ruddat. 1985. Development and enzyme activity of protein bodies in proteoplasts of tobacco root cells. *Histochemistry* 83:17-27.
3. Yaklich, R. W., E. L. Vigil and W. P. Wergin. 1986. Pore development and seed coat permeability in soybean. *Crop Sci.* 26:616-624.
4. Yaklich, R. W., W. P. Wergin and E. L. Vigil. 1986. Special secretory cells the soybean seed coat. *Protoplasma* 134:78-87.
5. Vigil, E. L. and E. F. Erbe. 1987. Warm and freeze-fracture of cotton seed radicles. *Proc. 45th Ann. Meeting of EMSA.* p. 984-985.
6. Fang, T. K., R. P. Donaldson and E. L. Vigil. 1987. Electron transport in purified glyoxysomal membranes. *Planta* 72:1-13.
7. Yaklich, R. W., E. L. Vigil and W. P. Wergin. 1987. Prevalence of the pit and anti-pit in the genus Glycine. *Proc. 45th Ann. Meeting of EMSA.* p. 986-987.
8. Yaklich, R. W., E. L. Vigil and W. P. Wergin. 1987. Changes in structure of pit and anti-pit in soybean seeds during germination and seedling development. *J. Seed Tech.* 11:151-157.
9. Yaklich, R. W., N. Chaney, E. L. Vigil and W. P. Wergin. 1988. Elemental composition of soybean seeds. *Proc. 46th Ann. Meeting of EMSA.* (accepted/in press)
10. Wergin, W. P., E. F. Erbe and E. L. Vigil. 1988. High vacuum evaporation and a cryostage to observe fractured yeast. *Proc. 46th Ann. Meeting of EMSA.* (accepted/in press).

B. Book Chapters:

1. Vigil, E. L., W. P. Wergin and M. N. Christiansen. 1985. Ultrastructural histochemistry with high voltage electron microscopy (HVEM). In: Advances in Microscopy. R. R. Cowden, editor. p. 243-263. (Symposium Invitation).
2. Vigil, E. L. 1986. Hypertension. "Searching, Teaching, Healing: American Indians and Alaskan Natives in Biomedical Research Careers. *Proc. of Conference* sponsored by NIH, Univ. Minnesota - Duluth, August 1-3, 1984. Futura Media Services, Inc. Mount Kisco, NY. (Symposium Invitation).



3. Vigil, E. L., R. L. Steere, M. N. Christiansen and E. Erbe. 1985. Structural changes in protein bodies of cotton radicles during seed maturation and germination. In: Botanical Microscopy. A. W. Robards, editor. Ch. 13, p. 311-334. (Symposium Invitation).
4. Vigil, E. L., T. K. Fang and R. P. Donaldson. 1987. Isolation of glyoxysomes. In: Methods in Enzymology: Plant Cell Membranes, Vol. 148: 505-516. R. Douce and L. Packer, editors. (Invited chapter).

C. Other Publications and Abstracts:

1. Vigil, E. L. and M. N. Christiansen. 1985. Development of storage organelles in cotton seed radicle. Proc. 39th Beltwide Cotton Physiol. Conf., p. 46. New Orleans, LA. (Abstract).
2. Vigil, E. L., R. Rowland and M. N. Christiansen. 1985. Protein body development in cotton seed radicles. Plant Physiol. 77:s121. (Abstract).
3. Yaklich, R. W., W. P. Wergin and E. L. Vigil. 1985. Unique cells in the soybean seed coat opposite the cotyledonary pit. Plant Physiol. 77:s121. (Abstract).
4. Yaklich, R. W., W. P. Wergin and E. L. Vigil. 1986. Possible route for photosynthate partitioning in soybean seeds. Mid-Atlantic Soybean Researchers Conference, p. 6. Beltsville, MD. (Abstract).
5. Yaklich, R. W., W. P. Wergin and E. L. Vigil. 1986. Freeze fracture of secretory cells in soybean seed coat. Amer. Soc. Cell Biol. (Abstract).
6. Vigil, E. L., R. Rowland and M. N. Christiansen and L C Frazier. 1986. Effects of nutritional stress on protein body formation in radicles of developing cotton seeds. Proc. 40th Beltwide Cotton Physiology Conference, p. 60. Las Vegas, NV. (Abstract).
7. Vigil, E. L., R. Rowland, E. Erbe and M. N. Christiansen. 1986. Effect of defoliation stress on protein body development in cotton seed radicles: Impact on seed quality and seedling growth. J. Histochem. Cytochem. 34:1072. (Abstract).
8. Vigil, E. L., L C Frazier and R. A. Rowland. 1988. Field and greenhouse test systems for studying effects of nutritional stress on embryogenesis in cotton. Proc. 42nd Beltwide Cotton Physiology Conference. New Orleans, LA. (Abstract - in press).



9. Vigil, E. L., L C Frazier and E. F. Erbe. 1988. Impact of nutritional stress on lipid and protein loading in radicles of cotton embryos. Proc. 42nd Beltwide Cotton Physiology Conference. New Orleans, LA. (Abstract - in press).
10. Vigil, E. L., R. P. Donaldson and R. A. Rowland. 1988. Effects of nutritional stress on lipid and protein loading in radicles of cotton embryos. Proc. 42nd Beltwide Cotton Physiology Conference. New Orleans, LA. (Abstract - in press).
11. Vigil, E. L., W. P. Wergin, N. Chaney, A. L. Fleming and R. P. Donaldson. 1988. Protein and mineral content of protein bodies in cotton radicles. Proc. 42nd Beltwide Cotton Physiology Conference. New Orleans, LA. (Abstract - in press).
12. Vigil, E. L., L C Frazier and C. Pooley. 1988. Image analysis of protein body development in radicles of cotton. Proc. International Congress on Histochemistry and Cytochemistry. Washington, D.C. - August. (Abstract - in press).





- I. Name: William P. Wergin  
Title: Supervisory Plant Physiologist  
Grade: GM-15
- II. CRIS Project No: 1209-23000-008 (90%)  
Title: Biophysical and biochemical properties of membranes  
in plant responses to environmental stress  
CRIS Project No: 1209-23000-007 (10%)  
Title: Role of light, temperature and water stress on the  
chloroplast antioxidant system
- III. Current Research Objectives, Plans and Accomplishments
- A. Objective: Utilize transmission and scanning electron microscopy to  
investigate structure-function relationships that result from  
environmental and biotic stress on plants.
- B. Plans: New techniques associated with EM have recently been  
acquired by the Electron Microscopy Unit. These techniques include  
a cryostage which allows one to examine with the scanning electron  
microscope frozen biological samples that were not subjected to  
chemical fixation, dehydration and critical point drying; energy  
dispersive X-ray microanalysis, which allows an investigator to  
obtain qualitative and semi-quantitative data on the elemental  
composition of a sample; and image analysis, which allows one to  
obtain statistical data on the structural composition of specimens.  
The use and applications of these techniques are being studied and  
evaluated for application to biological studies.
- C. Accomplishments:
1. Used cryostage on conventional scanning electron microscope.  
Showed that high vacuum evaporator can be used to shadow frozen  
tissue. Resolution obtainable, which is several-fold better than  
the conventional sputter coating technique that is commonly used,  
allows investigators to observe some macromolecular membrane  
structure on fresh, frozen, fractured tissue.
  2. Examined Solanum nigrum isogenic biotypes that were resistant and  
susceptible to the herbicide atrazine. Study showed that  
chloroplasts from the resistant strain accumulated less starch  
than those from the susceptible (0.17 vs. 1.23 grains/section)  
and that grana lamellae in resistant chloroplasts were 20% longer  
than those from susceptible organelles. Study indicates that the  
single amino acid transversion in the psb-A gene which codes for  
the 32 Kd protein also alters chloroplast structure-function  
relationships.
  3. Applied new instrument, low voltage field emission scanning  
electron microscopy, to biological investigations. Showed that  
little or no coating allows resolution of biological structures  
that were not previously seen or described.
- IV. Cooperators:
1. D. T. Krizek, M. N. Christiansen, C. Caldwell, R. Howell, C. D. Foy,  
Plant Stress Laboratory, ARS, Beltsville, MD



2. R. W. Yaklich, Germplasm Quality and Enhancement Lab, ARS, Beltsville, MD
3. J. R. Lichtenfels, Biosystematics Parasitology Lab, ARS, Beltsville, MD
4. R. L. Steere, Virology Lab, ARS, Beltsville, MD
5. E. L. Vigil, Department of Horticulture, University of Maryland, College Park, MD
6. F. E. Callahan, A. K. Mattoo, Plant Hormone Lab, ARS, Beltsville, MD.
7. J. B. St. John, H. A. Norman, Weed Science Lab, ARS, Beltsville, MD
8. B. Y. Endo, R. M. Sayre, Nematology Lab, ARS, Beltsville, MD
9. R. H. Zimmerman, Fruit Lab, ARS, Beltsville, MD

V. Curriculum Vitae:

A. Educational History:

1960-1964 - University of Wisconsin; major, Genetics; minor, Horticulture; BS 1964.

1964-1970 - University of Wisconsin; major, Botany; minor, Genetics; Ph.D. 1970.

1977 - Personnel Management, BMGT 360, University of Maryland, 3 credits.

1981 - Completed Personnel Management's Executive Seminar "Management Development Seminar" at Kings Point, New York, 80 hours.

B. Post-graduate Employment History:

1970-1972 GS-12 Research Morphologist, Nature of Resistance to Disease and Insect Attack Investigations Unit, Beltsville, Maryland

1972-1974 GS-12 Research Botanist, Weed Science Laboratory, USDA-ARS, Stoneville, Mississippi

1974-1979 GS-12/13 Research Microbiologist, Split appointment: Reproduction Laboratory and Nematology Laboratory, USDA-ARS, Beltsville, Maryland

1979-1980 GS-14 Research Cytologist, Split appointment: Nematology Laboratory and Cell Culture and Nitrogen Fixation Laboratory, USDA-ARS, Beltsville, Maryland.



1980-Present. GS-14/15 Supervisory Plant Physiologist, Research Leader, Plant Stress Laboratory, USDA-ARS, Beltsville, Maryland.

C. Society Offices Held (1986 - 88)

1. Gamma Sigma Delta, University of Maryland, National Capital Area Chapter, Secretary, 1987 - Present
2. Chesapeake Society of Electron Microscopy, Treasurer, 1987 - Present

D. Membership in Professional Societies:

American Association for the Advancement of Science

American Society of Cell Biology

American Society of Plant Physiologists

Botanical Society of America

Chesapeake Society of Electron Microscopy

Electron Microscopy Society of America

Gamma Sigma Delta

Helminthological Society of Washington

Microbeam Analysis Society

New York Academy of Sciences

Royal Microscopical Society (Fellow)

Sigma Xi

Society of Nematologists

Washington Academy of Sciences (Fellow)

E. Awards

Elected to membership Sigma Xi, 1970.

September 30, 1974. Dimond Award from the Botanical Society of America. This award was given to distinguished young scientists (under 35) to encourage their participation in the XII International Botanical Congress, Leningrad, Russia. 1975.

Awarded a Competitive Research Grant (\$65,000) for 1979-1981. Principal Investigator.



Awarded a Binational Agricultural Research and Development (BARD) Grant (\$180,000) for 1980-1983. Co-Principal Investigator.

Elected Fellow, Washington Academy of Sciences, 1985.

Elected Fellow, Royal Microscopical Society, 1987.

## VI. Publications:

### A. Peer Reviewed

1. Krizek, D. T., W. P. Wergin, Semenluk, P. Morphological and physiological properties of poinsettia leaves and bracts in relation to sulfur dioxide sensitivity. *Environ. Exp. Bot.* 25:165-173. 1985.
2. Vigil, E. L., R. L. Steere, W. P. Wergin and M. N. Christiansen. Structure of plasma membranes on radicles from cotton seeds. *Protoplasma.* 129:168-177. 1985.
3. Wergin, W. P. Interactions between spermatozoa and the crypts, cilia and mucus of the cervix in the ewe. *SEM.* 1985; III pp 1191-1199.
4. Wergin, W. P. Three-dimensional imagery and quantitative analysis of SEM studies of nematodes. *Agricult. Ecosyst. Environ.* 12:317-334. 1985.
5. Yaklich, R. W., W. P. Wergin and E. L. Vigil. Unique secretory cells in the soybean seed coat. *Protoplasma.* 134:78-87. 1986.
6. Yaklich, R. W., E. L. Vigil and W. P. Wergin. Pore development and seed coat permeability in soybean. *Crop Sci.* 26:616-624. 1986.
7. Lichtenfels, J. R., P. A. Pilitt and W. P. Wergin. Fine structure of the cuticle during development of the heartworm, Dirofilaria immitis, in dogs. *Proc. Helminthol. Soc. Wash.* 54:133-140. 1987.
8. Yaklich, R. W., E. L. Vigil and W. P. Wergin. Changes in structure of pit and antipit in soybean seeds during germination and seedling development. *J. Seed Tech.* 11:151-157. 1987.
9. Yaklich, R. W., E. L. Vigil and W. P. Wergin. Prevalence of the pit and antipit in the genus Glycine. *Proc. 45th Ann. Meeting EMSA.* pp. 986-987. 1987.
10. Endo, B. Y. and W. P. Wergin. Ultrastructure of the second stage juvenile of the root-knot nematode, Meloidogyne incognita. *Helminth. Soc. of Wash.*







11. Pasqualetto, P. L., R. H. Zimmerman and W. P. Wergin. Number of stomates, leaf thickness, palisade cell length and width and  $K^+$ ,  $Ca^{++}$ , and  $Mg^{++}$  leaf content in relation to vitrification of 'Gala' apple in vitro. Acta Horticulture. In Press.
12. Sayre, R. M., M. P. Starr, A. M. Golden, W. P. Wergin and B. Y. Endo. Diversity in the Pasteuria penetrans group of mycelial and endospore-forming bacteria, emphasizing populations parasitic on species of Protylechus and Meloidogyne. Helm. Soc. Wash. In Press.
13. Wergin, W. P., P. F. Bell and R. L. Chaney. Use of SEM, X-ray analysis and TEM TO localize  $Fe^{+3}$  reduction in roots. Proc. Elect. Micro. Soc. Amer. 1988. In Press.
14. Wergin, W. P., E. F. Erbe and E. L. Vigil. High vacuum evaporation and a cryostage to observe fractured yeast. Proc. Elect. Micro. Soc. Amer. 1988. In Press.
15. Wergin, W. P., R. M. Sayre and T. W. Reilly. Low voltage field emission electron microscopy applications in nematology. Proc. Elect. Micro. Soc. Amer. 1988. In Press
16. Yaklich, R. W., N. Chaney, E. L. Vigil and W. P. Wergin. Elemental composition of soybean seeds. Proc. Elect. Micro. Soc. Amer. 1988. In Press

#### B. Book Chapters:

1. Vigil, E. L., W. P. Wergin and M. N. Christiansen. Ultrastructural histochemistry with the highvoltage electron microscope (HVEM). In Adv. in Microscopy. pp 243-263. R. R. Cowden, ed. Alan R. Leiss Pub. 1985.
2. Norman, H. A., J. B. St. John, F. E. Callahan, A. K. Mattoo and W. P. Wergin. Lipid molecular species composition of granal and stromal lamellae. Metabolism Structure and Function of Plant Lipids. P. K. Stumpf, J. B. Mudd and W. D. Nes, eds. pp 192-195. Plenum Publ. Corp. 1987.
3. Wergin, W. P. Current trends and future developments in electron microscopy. Beltsville Symp. XI. In Press. Invited Chapter.
4. Wergin, W. P. and C. Pooley. Photographic and interpretive artifacts. Book Chapter. Invited. In Press.

#### C. Other Publications and Abstracts:

1. Krizek, D. T., Mirecki, R. M., Wergin, W. P. and Sicher, R. C. Physiology and ultrastructure of coleus leaves grown under LPS and SWF lamps at equal PPFD. Photochem. Photobiol. 39:1105. 1985.



2. Lichtenfels, J. R., P. A. Pilitt and W. P. Wergin. Cuticular changes in developmental stages of Dirofilaria immitis in the dog. Program and Abst. Amer. Soc. Parasitology. Athens Geo. p. 44. 1985
3. Yaklich, R. W., E. L. Vigil and W. P. Wergin. Development of pores and the impermeable seed coat in soybean. Mid Atl. Soybean Res. Conf., Beltsville. 1985
4. Yaklich, R. W., W. P. Wergin and E. L. Vigil. Unique secretion cells in the soybean seed coat opposite the cotyledonary pit. Plt. Physiol. 77:121. 1985
5. Yaklich, R. W., W. P. Wergin and E. L. Vigil. Unique cells in the soybean seed coat. Northeastern Agron. Meet., Amherst. 1985
6. Yaklich, R. W., E. L. Vigil and W. P. Wergin. Development of the soybean seed coat. Assoc. Office Seed Analysts Meet., Richmond, VA. 1985
7. Wergin, W. P., H. A. Norman, J. B. St. John and A. K. Mattoo. Ultrastructural and biochemical differences related to atrazine resistance in Solanum nigrum. Proc. XI. Int. Cong. on Electron Microsc., Kyto. p. 2553. 1986
8. Yaklich, R. W., W. P. Wergin and E. L. Vigil. Freeze fracture of secretory cells in soybean seed coat. Amer. Soc. Cell Biol. 1986
9. Sayre, R. M., W. P. Wergin, B. Y. Endo and M. P. Starr. Ultrastructural comparison of Pasteuria penetrans, a bacterial parasite of Meloidogyne incognita, and related parasite of Pratylenchus brachyurus. J. Nematology. 1987
10. Endo, B. Y. and W. P. Wergin. Fine-structure of the second-stage larvae of the root-knot nematode, Meloidogyne incognita. J. Nematology. (Abstract) In Press.
11. Endo, B. Y. and W. P. Wergin. Ultrastructure of the second-stage larvae of the root-knot nematode, Meloidogyne incognita. Int. Cong. Plant Pathol. In Press.
12. Sayre, R. M., W. P. Wergin, T. Nishizawa and M. P. Starr. Comparison of the fine structure of Pasteuria penetrans from Meloidogyne incognita with a related bacterium parasitizing Heterodera glycines. J. Nematology. In Press
13. Wergin, W. P. and R. M. Sayre. Applications of low voltage field emission SEM in nematology. J. Nematology. In Press.
14. Howell, R. K., W. P. Wergin and N. Chaney. Mineral distribution in peanuts as detected by SEM-ED X-ray analysis. Proc. APRES. In Press.



## Electron Microscope Facility

Building 177B, BARC-East

### I. Personnel

William P. Wergin

Eugene L. Vigil

Norita Chaney

Eric Erbe

L. C. Frazier

Gretchen Kaminski

Chris Pooley

### II. Instrumentation

#### A. Scanning Electron Microscopy

1. Hitachi 530 with Kevex energy dispersive x-ray analysis.

2. Hitachi 570 with Emscope cryostage.

#### B. Transmission Electron Microscopes

1. 2-Hitachi 500's

#### C. Cambridge Image Analyzer Model 970

#### D. Freeze-Etch and Freeze-Fracture Equipment



### III. Scientists Who Have Interacted With EM Facility 1986-1988

#### A. BARC Scientists

L. Batra	AO	
R. Chaney	AEQI	SMSL
J. St. John	AEQI	WSL
P. Allen	API	PDDL
R. Fayer	API	RPDL
R. Lichtenfels	API	PCDL
M. Bakst	ASI	APL
E. Robel	ASI	APL
A. Guidry	ASI	MSML
M. Paape	ASI	MSML
L. Johnson	ASI	RL
R. Wall	ASI	RL
M. Faust	HSI	FL
J. Maas	HSI	FL
J. Stapleton	HSI	FL
R. Zimmerman	HSI	FL
J. Neal	HSI	FNC
S. Batra	IIBIII	SEL
C. Gunn	PGGI	PETL
E. Herman	PGGI	PMGL
R. Yaklich	PGGI	GQEL
G. Beinhart	PPHI	PSL
A. Fleming	PPHI	PSL
R. Howell	PPHI	PSL
E. Lee	PPHI	PSL
M. Millard	PPHI	PSL
A. Mattoo	PPHI	PHL
R. Warmbrodt	PPHI	PPL
A. M. Golden	PPI	NL
W. Nickle	PPI	NL
R. Sayre	PPI	NL
J. Kaper	PPI	PVL
P. Martin	PPI	IPL

#### B. Other Scientists

E. Ashworth	AFRS
R. Bjork	ARS Info. Services
K. Corey	U.Md.
A. Hirsh	Red Cross
I. H. Mather	U.Md.
C. Meszoely	Northeastern Univ.
C. Ronning	U.Md.
T. Takahashi	Red Cross
E. Terrell	U.Md.
L. Thomas	U.Md.
E. Vigil	U.Md.
M. Wheeler	FSIS
K. Whittle	APHIS
M. Wisniewski	AFRS
T. Yaegashi	Japan





### III. Publications - 1985 - 1988

Bakst, M.R. Anatomical basis of sperm-storage in the avian oviduct. 1987. Scan. Micros., Vol. 1, No. 3, pp. 1257-1266.

Bakst, M.R. Embryonic Development of the chicken external cloaca and phallus. 1986. Scan. Elec. Micros. II, pp. 653-659.

Bakst, Murray R. and Cecil, Helene C. Embryonic development of the turkey male genitalia. 1986. Poultry Science 65:1623-1630.

Endo, B. Y. and W. P. Wergin. Ultrastructure of the second-stage juvenile of the root-knot nematode, Meloidogyne incognita. Helminth. Soc. of Wash.

Endo, B. Y. and W. P. Wergin. Fine-structure of the second-stage larvae of the root-knot nematode, Meloidogyne incognita. J. Nematology. (Abstract) In Press.

Endo, B. Y. and W. P. Wergin. Ultrastructure of the second-stage larvae of the root-knot nematode, Meloidogyne incognita. Int. Cong. Plant Pathol. In Press.

Fang, T.K., Donaldson, R.P. and Vigil, E.L. Electron transport in purified glyoxysomal membranes from castor-bean endosperm. 1987. Planta Vol. 172, pp 1-13.

Guidry, A.J., Squiggins, K., Kavlich, M. and Erbe, E.F. A model for studying Staphylococcus aureus capsule 27th Annual Meeting of the National Mastitis Council, Inc. p. 104. 1988(Abstract)

Hoberg, Eric P., Zimmerman, Gary L. and J. Ralph Lichtenfels. First report of nematodirus battus (Nematoda=Trichostrongyloidea) in North America; redescription and comparison to other species. 1986. Proc. Helminthol. Soc. Wash. 53 (1), pp 80-88.

Howell, R. K., W. P. Wergin and N. Chaney. Mineral distribution in peanuts as detected by SEM-ED X-ray analysis. Proc. APRES. In Press.

Jaffe, H., Hayes, D.K., Amarnath, N. and Chaney, N. Preparation and scanning electron microscopy of microcapsules of abate. Dec 1986. Pesticides, Vol. XX, No. 12.

Kavlich, M.F., Erbe, E.F., Guidry, A.J. and Roberson, B.S. Description of a variant strain of Smith diffus on proceedings Federation American Society Experimental Biology 46(Number 4):1320 (Abstract) 1987.

Krizek, D. T., Mirecki, R. M., Wergin, W. P. and Sicher, R. C. Physiology and ultrastructure of coleus leaves grown under LPS and SWF lamps at equal PPFD. 1985. Photochem. Photobiol. 39:1105.

Krizek, D. T., W. P. Wergin, Semeniuk, P. Morphological and physiological properties of poinsettia leaves and bracts in relation to sulfur dioxide sensitivity. 1985. Environ. Exp. Bot. 25:165-173.



Lichtenfels, J. R., P. A. Pilitt and W. P. Wergin. Fine structure of the cuticle during development of the heartworm, Dirofilaria immitis, in dogs. 1987. Proc. Helminthol. Soc. Wash. 54:133-140.

Lichtenfels, J. R., P. A. Pilitt and W. P. Wergin. Cuticular changes in developmental stages of Dirofilaria immitis in the dog. Program and Abst. 1985. Amer. Soc. Parasitology. Athens Geo. p. 44.

Lichtenfels, J.R. and Pilitt, P.A. Eustrorgylides sp. (Nematoda: Dioctophymatoidea): differentiation of third- and fourth-stage larvae from Killifish, Fundulus sp., collected in Chesapeake Bay Area, USA. 1986. Proc. Helminthol. Soc. Wash. 53(1), pp. 144-148.

Lichtenfels, J.R., Pilitt, P.A. and LeJambre, L.F. Cuticular ridge patterns of haemonchus contortus and Haemonchus placei (Nematoda: Trichostrongyloidea). 1986. Proc. Helminthol. Soc. Wash. 53(1), pp 94-101.

Meszoely, C.A.M., Erbe, E.F., Steere, R.L., Trosper, J. and Beaudoin, R.L. Plasmodium calciparum: freeze fracture of the gametocyte. 1987. Pellicular Complex Experimental Parasitology 64, pp. 300-309.

Meszoely, Ch. A.M., Erbe, E.F., and Beaudoin, R.L. Comparison of the pellicles of different stages of the malaria parasites using freeze-fracture techniques. 1986. Symposia Biologica Hungarica 32:501-505. Proceedings of the 1st International Conference of Hungary on Protozoology, published by Hungarian Academy of Science.

Neal, John W., Jr. Salient vestiture and morphological characters of the pharate female bagworm, Thyridopteryx ephemaraeformis (Lepidoptera: Psychidae). 1986. An. Entomol. Soc. Am. 79:814-820.

Norman, H. A., J. B. St. John, F. E. Callahan, A. K. Mattoo and W. P. Wergin. Lipid molecular species composition of granal and stromal lamellae. Metabolism Structure and Function of Plant Lipids. 1987. P. K. Stumpf, J. B. Mudd and W. D. Nes, eds. pp 192-195. Plenum Publ. Corp.

Pasqualetto, P. L., R. H. Zimmerman and W. P. Wergin. Number of stomates, leaf thickness, palisade cell length and width and  $K^+$ ,  $Ca^{++}$ , and  $Mg^{++}$  leaf content in relation to vitrification of 'Gala' apple in vitro. Acta Horticulture. In Press.

Sayre, R. M., M. P. Starr, A. M. Golden, W. P. Wergin and B. Y. Endo. Diversity in the Pasteuria penetrans group of mycelial and endospore-forming bacteria, emphasizing populations parasitic on species of Prottylenchus and Meloidogyne. Helm. Soc. Wash. In Press.

Sayre, R. M., W. P. Wergin, B. Y. Endo and M. P. Starr. Ultrastructural comparison of Pasteuria penetrans, a bacterial parasite of Meloidogyne incognita, and related parasite of Pratylenchus brachyurus. J. Nematology. 1987

Sayre, R. M., W. P. Wergin, T. Nishizawa and M. P. Starr. Comparison of the fine structure of Pasteuria penetrans from Meloidogyne incognita with a related bacterium parasitizing Heterodera glycines. J. Nematology. In Press



Stapleton, J.J. Effects of postharvest chlorine and wax treatments on surface microflora of lime fruit in relation to citrus bacteriosis disease. *Plant Disease*, Vol. 70, No. 11, pp 1046-1048.

Takahashi, T., Hirsh, A., Erbe, E.F., Bross, J.B., Steere, R.L. and Williams, R.J. Vitrification of human monocytes. April 1986. *Cryobiology* Vol. 23:103-115.

Takahashi, T., Hirsh, A., Bross, J.B., Erbe, E.F., Williams, R.J. and Steere, R.L. Detailed visualization of the vitrification of human monocytes. 1986. *Cryobiology* 23:555 (Abstract). *Cryobiology Meeting at Augusta Georgia* June 17-20.

Takahashi, T., Hirsh, A., Erbe, E.F., Hammell, M.F., Steere, R.L. and Williams, R.J. Polymers protect monocytes from freezing injury through high temperature extracellular glass formation. 1986. *Cryobiology* 23:556 (Abstract). *Cryobiology Meeting at August Georgia* June 17-20.

Takahashi, T., Noji, S., Erbe, E.F., Steere, R.L., Kon, H. Cold shock hemolysis in human erythrocytes studied by spin probe method and freeze-fracture electron microscopy. Feb. 1986. *Biophysical Journal* Vol. 49:403-410.

Vigil, E. L. 1986. Hypertension. "Searching, Teaching, Healing: American Indians and Alaskan Natives in Biomedical Research Careers. Proc. of Conference sponsored by NIH, Univ. Minnesota - Duluth, August 1-3, 1984. Futura Media Services, Inc. Mount Kisco, NY. (Symposium Invitation).

Vigil, E. L. and Christiansen, M.N. 1985. Development of storage organelles in cotton seed radicle. Proc. 39th Beltwide Cotton Physiol. Conf., p. 46. New Orleans, LA. (Abstract).

Vigil, E. L. and Erbe, E.F. 1987. Warm and freeze-fracture of cotton seed radicles. Proc. 45th Ann. Meeting of EMSA. p. 984-985.

Vigil, E. L., Frazier, L.C. and Rowland, R.A. 1988. Field and greenhouse test systems for studying effects of nutritional stress on embryogenesis in cotton. Proc. 42nd Beltwide Cotton Physiology Conference. New Orleans, LA. (Abstract - in press).

Vigil, E. L., Frazier, L.C. and Erbe, E.F. 1988. Impact of nutritional stress on lipid and protein loading in radicles of cotton embryos. Proc. 42nd Beltwide Cotton Physiology Conference. New Orleans, LA. (Abstract - in press).

Vigil, E. L., Frazier, L.C. and Pooley, C. 1988. Image analysis of protein body development in radicles of cotton. Proc. International Congress on Histochemistry and Cytochemistry. Washington, D.C. - August. (Abstract - in press).

Vigil, E. L., Steere, R.L., Wergin, W.P. and Christiansen, M.N.. Structure of plasma membranes on radicles from cotton seeds. *Protoplasma*. 129:168-177. 1985.





- Vigil, E. L., Steere, R.L., Christiansen, M.N. and Erbe, E.F. 1985. Structural changes in protein bodies of cotton radicles during seed maturation and germination. In: Botanical Microscopy. A. W. Robards, editor. Ch. 13, pp 311-334. (Symposium Invitation).
- Vigil, E. L., Donaldson, R.P. and Rowland, R.A. Effects of nutritional stress on lipid and protein loading in radicles of cotton embryos. 1988. Proc. 42nd Beltwide Cotton Physiology Conference. New Orleans, LA. (Abstract - in press).
- Vigil, E. L., Rowland, R.A. and Christiansen, M.N. 1985. Protein body development in cotton seed radicles. *Plant Physiol.* 77:s121. (Abstract).
- Vigil, E. L., Rowland, R.A., Christiansen, M.N. and Frazier, L.C. 1986. Effects of nutritional stress on protein body formation in radicles of developing cotton seeds. Proc. 40th Beltwide Cotton Physiology Conference, p. 60. Las Vegas, NV. (Abstract).
- Vigil, E. L., Rowland, R.A., Erbe, E. and Christiansen, M.N. 1986. Effect of defoliation stress on protein body development in cotton seed radicles: Impact on seed quality and seedling growth. *J. Histochem. Cytochem.* 34:1072. (Abstract).
- Vigil, E. L., Fang, T.K. and Donaldson, R.P. 1987. Isolation of glyoxysomes. In: Methods in Enzymology: Plant Cell Membranes, Vol. 148: 505-516. R. Douce and L. Packer, editors. (Invited chapter).
- Vigil, E. L., W. P. Wergin and M. N. Christiansen. Ultrastructural histochemistry with the high voltage electron microscope (HVEM). 1985. In Adv. in Microscopy. pp 243-263. R. R. Cowden, ed. Alan R. Leiss Pub.
- Vigil, E. L., Wergin, W.P., Chaney, N., Fleming, A.L. and Donaldson, R.P. 1988. Protein and mineral content of protein bodies in cotton radicles. Proc. 42nd Beltwide Cotton Physiology Conference. New Orleans, LA. (Abstract - in press).
- Vigil, E. L., and Ruddat, M. 1985. Development and enzyme activity of protein bodies in proteoplasts of tobacco root cells. *Histochemistry* 83:17-27.
- Wergin, W. P. Current trends and future developments in electron microscopy. Beltsville Symp. XI. In Press. Invited Chapter.
- Wergin, W. P. Interactions between spermatozoa and the crypts, cilia and mucus of the cervix in the ewe. SEM. 1985; III pp 1191-1199.
- Wergin, W. P. Three-dimensional imagery and quantitative analysis of SEM studies of nematodes. *Agricult. Ecosyst. Environ.* 1985. 12:317-334.
- Wergin, W. P. and Pooley, C. Photographic and interpretive artifacts. Book Chapter. Invited. In Press.





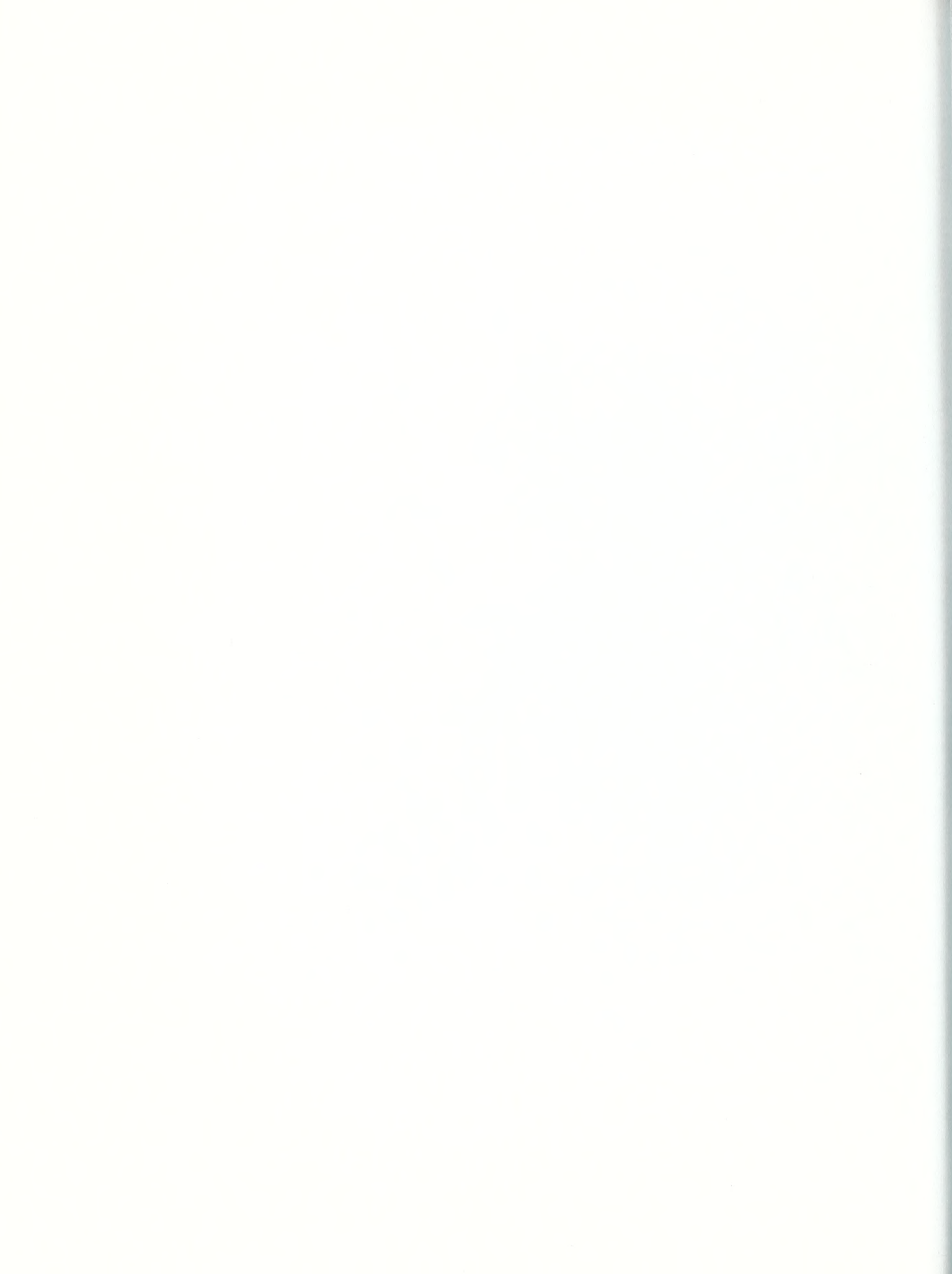
- Wergin, W. P. and Sayre, R.M. Applications of low voltage field emission SEM in nematology. J. Nematology. In Press.
- Wergin, W. P., Erbe, E.F. and Vigil, E.L. High vacuum evaporation and a cryostage to observe fractured yeast. Proc. Elect. Micro. Soc. Amer. 1988. In Press.
- Wergin, W. P., H. A. Norman, J. B. St. John and A. K. Mattoo. Ultrastructural and biochemical differences related to atrazine resistance in Solanum nigrum. Proc. XI. Int. Cong. on Electron Microsc., Kyoto. p. 2553. 1986
- Wergin, W. P., P. F. Bell and R. L. Chaney. Use of SEM, X-ray analysis and TEM to localize  $FE^{+3}$  reduction in roots. Proc. Elect. Micro. Soc. Amer. 1988. In Press.
- Wergin, W. P., R. M. Sayre and T. W. Reilly. Low voltage field emission electron microscopy applications in nematology. Proc. Elect. Micro. Soc. Amer. 1988. In Press
- Whittle, K. Garden, Weevil - Phlyctinus callosus Boheman. September 1986. APHIS, 81-49
- Yaklich, R. W., E. L. Vigil and W. P. Wergin. Pore development and seed coat permeability in soybean. Crop Sci. 26:616-624. 1986.
- Yaklich, R. W., E. L. Vigil and W. P. Wergin. Changes in structure of pit and antipit in soybean seeds during germination and seedling development. J. Seed Tech. 11:151-157. 1987.
- Yaklich, R. W., E. L. Vigil and W. P. Wergin. Prevalence of the pit and antipit in the genus Glycine. Proc. 45th Ann. Meeting EMSA. pp. 986-987. 1987.
- Yaklich, R. W., E. L. Vigil and W. P. Wergin. Development of pores and the impermeable seed coat in soybean. Mid Atl. Soybean Res. Conf., Beltsville. 1985
- Yaklich, R. W., E. L. Vigil and W. P. Wergin. Development of the soybean seed coat. Assoc. Office Seed Analysts Meet., Richmond, VA. 1985
- Yaklich, R. W., N. Chaney, E. L. Vigil and W. P. Wergin. Elemental composition of soybean seeds. Proc. Elect. Micro. Soc. Amer. 1988. In Press
- Yaklich, R. W., W. P. Wergin and E. L. Vigil. Unique secretory cells in the soybean seed coat. Protoplasma. 134:78-87. 1986.
- Yaklich, R. W., W. P. Wergin and E. L. Vigil. Unique secretion cells in the soybean seed coat opposite the cotyledonary pit. Plt. Physiol. 77:121. 1985
- Yaklich, R. W., W. P. Wergin and E. L. Vigil. Unique cells in the soybean seed coat. Northeastern Agron. Meet., Amherst. 1985
- Yaklich, R. W., W. P. Wergin and E. L. Vigil. 1986. Possible route for photosynthate partitioning in soybean seeds. Mid-Atlantic Soybean Researchers Conference, p. 6. Beltsville, MD. (Abstract).



Yaklich, R. W., W. P. Wergin and E. L. Vigil. 1986. Freeze fracture of secretory cells in soybean seed coat. Amer. Soc. Cell Biol. (Abstract).







NATIONAL AGRICULTURAL LIBRARY



1022577768

NATIONAL AGRICULTURAL LIBRARY



1022577768